DIAGNOSTIC ACCURACY OF ACOUSTIC RADIATION FORCE IMPULSE (ARFI) ELASTOGRAPHY OF THE LIVER FOR THE DETECTION AND STAGING OF LIVER FIBROSIS IN COMPARISON TO LIVER BIOPSY IN THALASSEMIC PATIENTS PRIOR TO BONE MARROW TRANSPLANTATION

A dissertation submitted in partial fulfillment of MD Radiodiagnosis (Branch VIII) examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai to be held in April 2016.

CERTIFICATE

This is to certify that the dissertation entitled "Diagnostic accuracy of Acoustic Radiation Forced Impulse (ARFI) Elastography for the detection and staging of liver fibrosis in comparison to liver biopsy in thalassemic patients prior to Bone Marrow Transplantation" is a bonafide original work of Dr. Priyanka Mohapatra submitted in partial fulfilment of the requirement for MD Radiodiagnosis (Branch VIII) Degree Examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai to be held in April 2016.

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DECLARATION

I, Dr. Priyanka Mohapatra, hereby declare that this dissertation entitled "Diagnostic accuracy of Acoustic Radiation Forced Impulse (ARFI) Elastography for the detection and staging of liver fibrosis in comparison to liver biopsy in Thalassemic patients prior to Bone Marrow Transplantation" is an original work done by me in partial fulfilment of the requirement for M.D Radio Diagnosis (Branch- VIII) Degree Examination of The Tamil Nadu Dr M.G.R Medical University, Chennai to be conducted in April, 2016.

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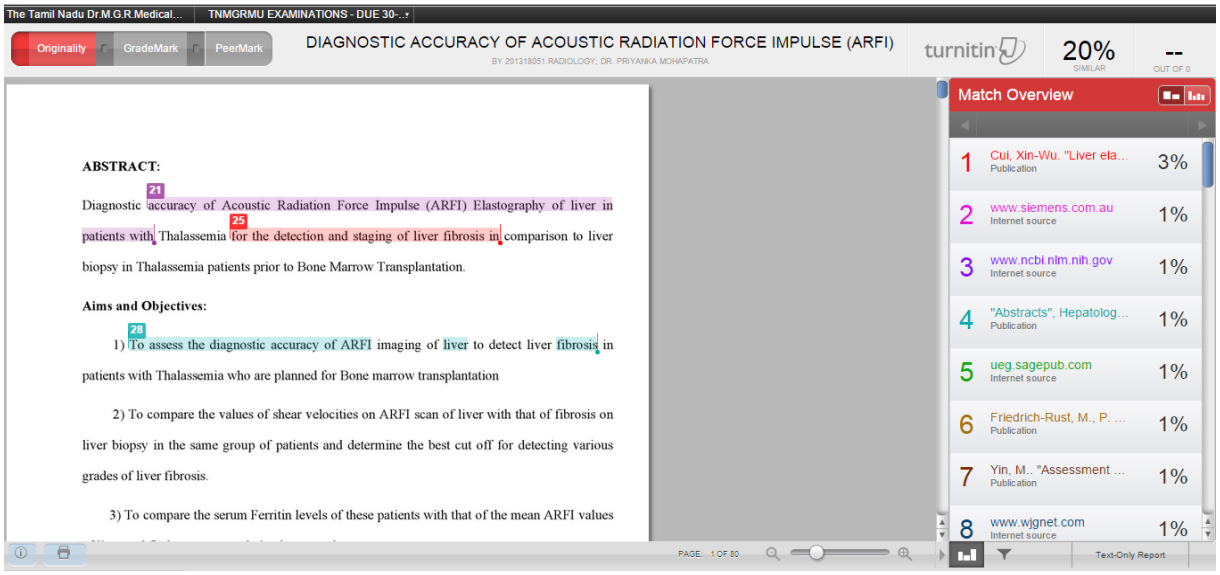
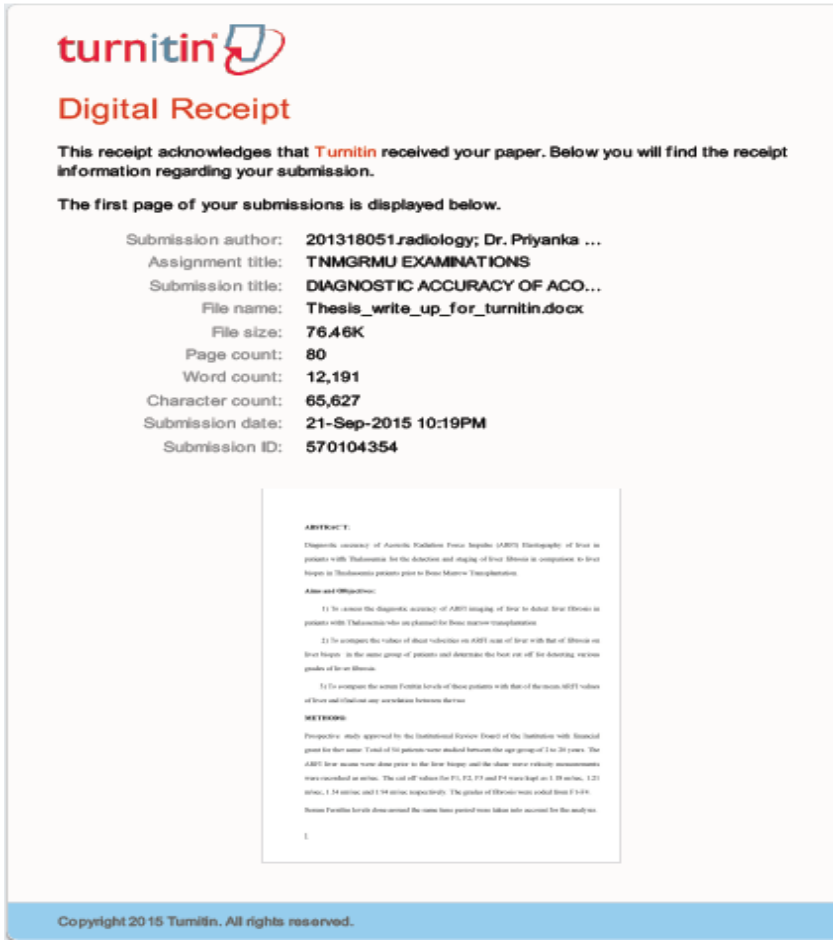
PLAGIARISM CERTIFICATE





ACKNOWLEDGEMENTS

This study could be carried out only due to the untiring efforts and hard work of many individuals. I wish to place in record my sincere appreciation and immense gratitude to them. To my guide and co-guide, Dr. Anu Eapen and Dr. Betty Simon for their continued support and guidance in performing this study.

Dr. Aby Abraham and all the doctors and nurses in department of Hematology who extended their continual support throughout these two years.

Special note of thanks to Dr. Banumathi Ramakrishna and Dr. Thomas Alex from the department of pathology who continually helped in assessing the liver biopsy samples and helped me throughout the process.

Mrs. Kavitha; statistician for all her help in the data analysis.

I am immensely thankful to all my Thalassemia patients (children) and their parents without whom this study would not have been possible.

My family, friends and colleagues for their love, constant support and encouragement.

I thank GOD for his abundant grace and mercy which is abounding.

ABBREVIATIONS:

ALT: Alanine aminotransferase

APRI: Aspartate aminotransferase (AST)-to-platelet ratio index

ARFI: Acoustic radiation force impulse

AST: Aspartate aminotransferase

AUROC: Area under receiver operator curve

BMI: Body mass index

CLD: Chronic liver disease

CHB: Chronic hepatitis B

CHC: Chronic hepatitis C

HBV: Hepatitis B virus

HCV: Hepatitis C virus

HIV: Human immunodeficiency virus

LR: Likelihood ratio

LS: Liver stiffness

MRE: Magnetic Resonance Elastography

NAFLD: Non alcoholic fatty liver disease

NPV: Negative predictive value

PPV: Positive predictive value

ROI: Region of interest

SWV: Shear wave velocity

TE: Transient elastography

VTQ: Virtual Touch Tissue Quantification

CONTENTS:

ABSTRACT:

Diagnostic accuracy of Acoustic Radiation Force Impulse (ARFI) Elastography of liver in patients with Thalassemia for the detection and staging of liver fibrosis in comparison to liver biopsy in Thalassemia patients prior to Bone Marrow Transplantation.

Aims and Objectives:

1) To assess the diagnostic accuracy of ARFI imaging of liver to detect liver fibrosis in patients with Thalassemia who are planned for Bone marrow transplantation

2) To compare the values of shear velocities on ARFI scan of liver with that of fibrosis on liver biopsy in the same group of patients and determine the best cut off for detecting various grades of liver fibrosis.

3) To compare the serum Ferritin levels of these patients with that of the mean ARFI values of liver and find out any correlation between the two

METHODS:

Prospective study approved by the Institutional Review Board of the Institution with financial grant for the same. Total of 54 patients were studied between the age group of 2 to 20 years. The ARFI liver scans were done prior to the liver biopsy and the shear wave velocity measurements were recorded as m/sec. The cut off values for F1, F2, F3 and F4 were kept as 1.18 m/sec, 1.21 m/sec, 1.54 m/sec and 1.94 m/sec respectively. The grades of fibrosis were coded from F1-F4.

Serum Ferritin levels done around the same time period were taken into account for the analysis.

Subsequently, the patients underwent liver biopsy; the samples of which were also studied and grades of fibrosis were given from F1-F6 based on the popular Ishak grading system for liver fibrosis assessment.

Results:

54 Thalassemia patients (37 male and 17 females) with a median age of 8.1 years (range 2-20 years) were studied. All 54 cases underwent liver biopsy. There was one insufficient liver biopsy sample hence this patient was not included in the statistical analysis. Keeping the cut offs of ≥ 1.18 m/sec, ≥ 1.21 m/sec, ≥ 1.54 m/sec and 1.94 m/sec on ARFI for F1, F2, F3 and F4 grades of fibrosis, the sensitivity and PPV of ARFI to detect the corresponding grades of fibrosis on liver biopsy was 89 % and 89% with a diagnostic accuracy of 81%; while the specificity and NPV value were 29% and 29% respectively. There was only 18% agreement between ARFI liver and fibrosis on liver biopsy which was not statistically significant ($p = 0.942$).

There was moderate correlation between the serum Ferritin values and the ARFI grades of fibrosis; however it did not show any linear increase in values. About 84.3 % of patients with serum ferritin values of >1000 ng / ml were distributed within the F2, F3 fibrosis grades of ARFI.

Conclusions:

ARFI elastography is a novel method in diagnosing liver fibrosis as compared to liver biopsy in children with Thalassemia. There was good correlation between liver stiffness measured by ARFI and grades of fibrosis on liver biopsy (using the ISHAK grading system of liver biopsy) as seen in this study with a sensitivity of 89%, positive predictive value of 89% and a diagnostic accuracy of 81%.

AIMS AND OBJECTIVES:

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JUSTIFICATION:

In patients with Thalassemia due to multiple blood transfusions there is increased iron overload in various organs of the body such as liver, heart, muscles, pancreas, pituitary etc. In liver it manifests as fibrosis. To assess fibrosis these patients are subjected to liver biopsy which is an invasive procedure and can cause many potential and life threatening complications.

Liver fibrosis can also be picked up by a new development in the field of ultrasonography called Acoustic Radiation Forced Impulse Imaging (ARFI) scan in which the elasticity of the liver tissue is assessed based on the ARFI shear wave propagation velocity in the tissue, measured as m/sec. The shear wave propagation velocity in the tissue under study is a quantitative indirect measure of the tissue stiffness. These numerical values hold potential promise of assessing liver fibrosis in comparison to liver biopsy.

Our aim is to measure liver stiffness using ARFI and to compare its performance in comparison to liver biopsy in detecting and staging liver fibrosis.

If this non-invasive test can pick up the liver fibrosis effectively than in future it could replace the invasive method; liver biopsy.

LITERATURE REVIEW:

Introduction to Thalassemia:

Various methods to assess liver fibrosis and gold standard:

Liver biopsy and its various Histopathological grades in assessing fibrosis:

Various methods to assess liver fibrosis by means of non-invasive methods (serum and imaging):

Introduction to non-invasive tests to assess liver fibrosis by Ultrasound Elastography:

Acoustic Radiation Force Impulse (ARFI) Elastography:

- Principle and protocol for ARFI elastography of liver
- How is ARFI done?
- Diagnostic accuracy of ARFI in staging liver fibrosis
- Reproducibility of ARFI elastography
- Factors influencing ARFI elastography measurements
- Limitations of the ARFI study(especially in children)
- ARFI elastography vs Transient elastography (TE) and its advantages over Transient Elastography
- MRI Elastography and MRI T2* Imaging in assessment of Iron Overload in Thalassemic children

Assessment of serum Ferritin in Thalassemic children and its relevance:

Introduction to Thalassemia:

Adult human hemoglobin is a made up of two alpha globin chains and two beta globin chains forming a tetramer.

When one or more of this globin chain production is reduced or absent then the rationis disrupted giving various spectrum of Thalassemia.

Four types are described namely:

1. **Alpha or beta thalassemia minor**: Usually asymptomatic adults who may have minor degree of anemia of the microcytic, hypochromic type.

2. **Thalassemia intermedia**: Can have variable mutation of hemoglobin in the same patient and is of intermediate severity

3. and 4. **Beta thalassemia major and alpha thalassemia major:** Lifelong transfusion dependence in beta thalassemia patients and alpha thalassemia is incompatible with extrauterine life.

BETA THALASSEMIA MAJOR

Due to decreased production of beta globin chains and excess production of alpha globin chains which are incapable of forming tetramers this leads to various clinical manifestations due to alpha chain precipitation within the cell.

The homozygous beta thalassemia patients are the ones severely affected with excess amount of alpha globin chains; however the heterozygotes have lesser degree of anaemia and clinical symptoms.

Hemoglobin (HbF) comprising 2 alpha globins and 2 gamma globins is produced at the time of birth and so infants with severe beta thalassemia major are well at birth.

But by around 6-12 months of age the signs and symptoms of beta Thalassemia major start to appear when beta globin chains replace the gamma chains and form the adult hemoglobin. There is lack of beta globin chain production which ultimately leads these children to develop anemia and its various hemolytic effects in these children.

The initial manifestations in infancy are pallor, irritability, growth retardation, jaundice etc which are a due to the hemolytic effects. As there is ineffective erythropoiesis bony changes and skeletal abnormalities soon set in. About 80% of the less than 5 year old children who are left without any treatment will succumb to the disease and the rest of them will usually die due to failure to thrive or mostly by its direct long term consequences such as severe anemia.

The most severe manifestations of Beta Thalassemia Major are described in detail below; most of which are unusually seen in developed countries with good medical care facilities.

The various manifestations can be classified as follows:

1) Effects due to anemia and chronic hemolytic effects.

2) Effects due to iron overload (transfusion related)

Effects of anemia include failure to thrive, hepatosplenomegaly, bony abnormalities etc.(1)

Complications due to iron overload can involve various organs such as heart and can produce dilated myocardiopathy; myocardial siderosis being the most important life threatening one in this group of transfusion overload patients. In the liver it can lead to fibrosis and subsequently to cirrhosis. It can also cause diabetes mellitus, parathyroid insufficiency and hypogonadism due to its effects on the endocrine organs. Other complications due to transfusion dependence are chronic hepatitis (due to hepatitis B and C viruses), HIV infection.

Osteoporosis and venous thrombosis are other complications due to transfusion overload.

The major cause of the deaths in these patients with beta Thalassemia major is myocardial siderosis.

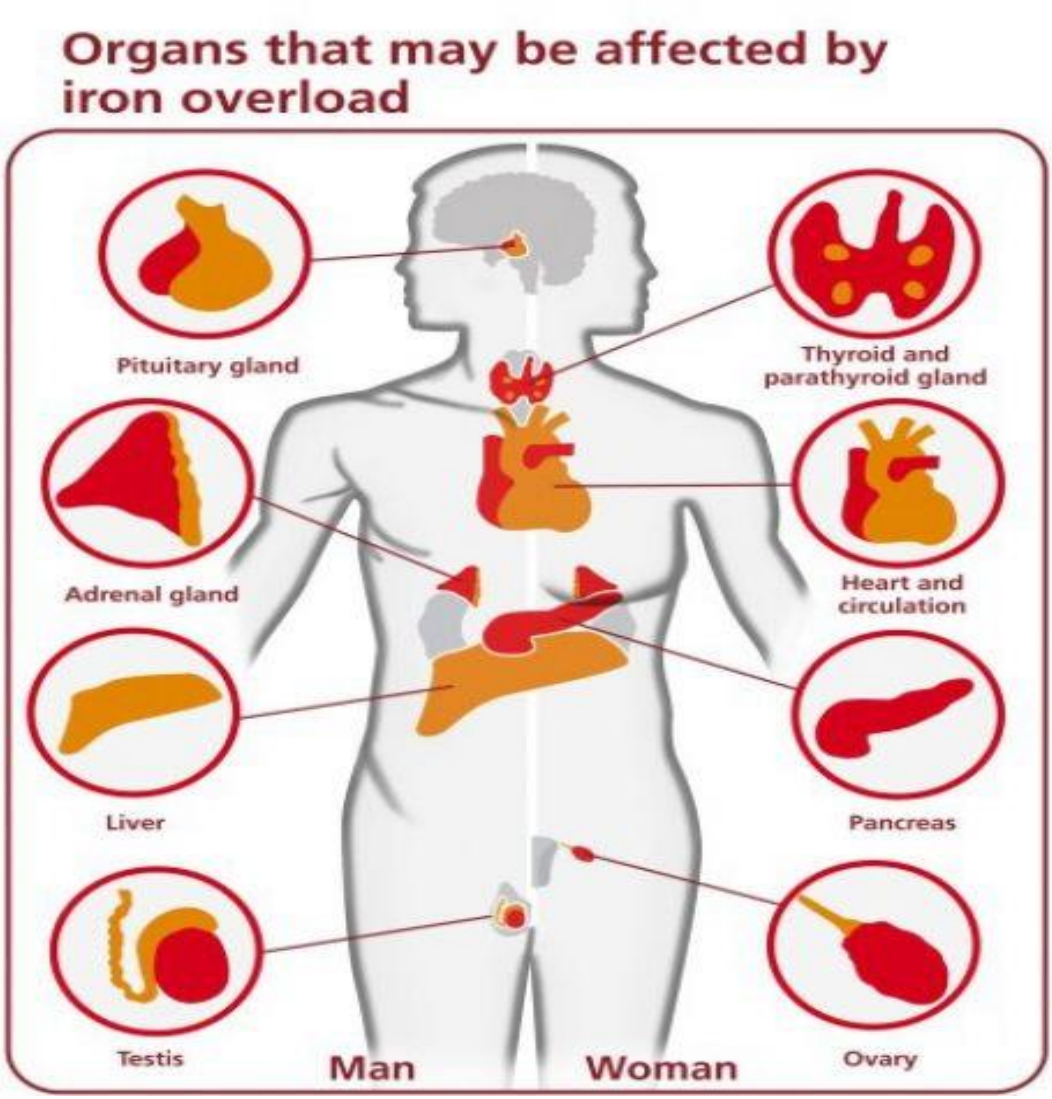


Diagram 1: Effects of iron overload in various organs in Thalassemia. (2)

Liver fibrosis is a common pathway and a consequence to various liver pathologies; the various causes being hereditary, metabolic, autoimmune, viral and toxin-mediated liver disease. All the above processes lead to hepatocyte dysfunction which in turn leads to extracellular matrix expansion and associated deformation of the liver architecture. This in turn leads to the grave situation of portal hypertension and finally

liver cirrhosis(3). Therefore it is very important to define the degree of liver fibrosis as it is very important for the disease prognosis and in making decision regarding treatment for these groups of patients with fibrosis / cirrhosis.

Methods to assess liver fibrosis and gold standard:

There are various methods available for the assessment of liver fibrosis; liver biopsy being the current available gold standard.

The tests can be classified as:

a) Invasive:

LIVER BIOPSY:

The current gold standard for assessment of liver fibrosis is histopathological examination of the liver biopsy specimen (4). However, being an invasive test it has many limitations and it may be associated with complications, hence is usually not welcomed by patients.

Also only a small portion of the liver is sampled by biopsy , making it susceptible to sampling variation and it can lead to inter- and intraobserver variability leading to misinterpretations of results (5).

Liver biopsy has an important role in the diagnosis of intrahepatic portal hypertension and has limited role in the prehepatic and post hepatic causes of portal hypertension. In

patients with portal vein thrombosis, liver biopsy is indicated when there is cirrhosis or nodular regenerative hyperplasia.

ROUTES OF LIVER BIOPSY:

1. Percutaneous biopsy

2. Transjugular biopsy

3. Laparoscopic biopsy

4. Fine needle aspiration or biopsy or under ultrasonography or computed tomography.

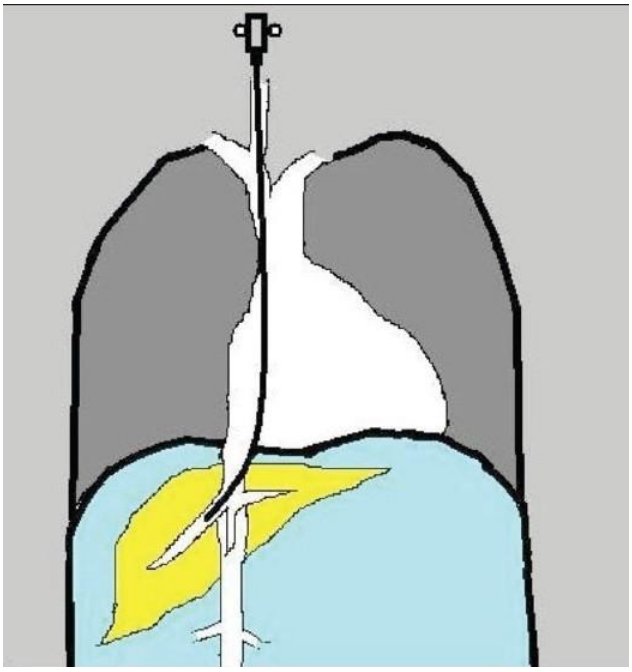


Diagram 2: The position of the Transjugular liver biopsy needle in the liver.(6)

ADEQUATE LIVER BIOPSY SPECIMEN:

A 1.5 cm long–1.4 mm wide sample with ~ 11 complete portal tracts is recommended to ensure reliable grading and staging. Each histopathological report should also give a

clear picture about the limitations of histological interpretation, number of complete portal tracts and the size of specimen which should be easily available for the clinician during the assessment of the patient.(7)

SCORING SYSTEMS OF LIVER FIBROSIS ON HISTOPATHOLOGY(8)**:**

Various systems are used to classify fibrosis into various stages. This is based on the amount of collagen staining on the liver biopsy specimen. The various staging systems used are the METAVIR score, Histology activity index (HAI: Knodell score) and the Ishak modification of the HAI score. Each of the score has its own advantages and disadvantages.

The METAVIR scoring method is a semiquantitative classification system which looks at necroinflammatory activity and the degree of liver fibrosis. It has good inter and intra-observer reproducibility. Fibrosis is assessed on a scale of 0 to 4. Clinically significant fibrosis is that which is greater than F2.

The METAVIR scoring is as follows:

F0- no fibrosis

F1- Stellate enlargement of portal tract however without septa formation

F2- Enlargement of portal tract with rare/few septa formation

F3- Numerous septa formation

F4- Cirrhosis

According to the Ishak scoring system (modified Knodell score) there are six grades of fibrosis ranging from F1-F6 which are convertible to the METAVIR scoring system which grades fibrosis from F1-F4.

Ishak scoring system is better than METAVIR, because in the latter only interface hepatitis and lobular necrosis determine the grade of activity, whereas in Ishak system, portal infiltrate and confluent necrosis are also included along with the two previous parameters.

The Ishak Scoring system of liver fibrosis is as follows:

F0- No evidence of fibrosis

F1- Fibrous expansion of some portal areas, with or without short fibrous septa

F2- Fibrous expansion of most portal areas, with or without short fibrous septa

F3- Fibrous expansion of most portal areas, with occasional portal to portal (P-P) bridging

F4- Fibrous expansion of portal areas with marked bridging (portal to portal as well portal to central)

F5- Marked bridging (portal to portal as well portal to central) with occasional nodules (incomplete cirrhosis)

F6- Cirrhosis; probable or definite

The scores of Ishak and METAVIR can be compared and be converted from Ishak to METAVIR as illustrated below (8).

Ishak Score; Fibrosis 0-6	METAVIR Score; Fibrosis 0-4
0	0
1-2	1
3	2
4-5	3
6	4

LIMITATIONS OF LIVER BIOPSY:

- It is an invasive procedure which requires inpatient care and post procedural monitoring for 24 hours.
- Sampling error: Liver fibrosis is not uniform throughout the liver. Liver biopsy samples only 1/ 50,000th part of the liver parenchyma; hence it may underestimate the degree and extent of liver fibrosis.
- Liver biopsy specimen may be inadequate or have fragmented specimens.
- Inter and intra-observer variability in interpretation especially in macronodular cirrhosis.

- Complications rate of 7% including hemorrhage, biliary leak or fistula.
- Patients can have pain in 20% of cases and few of them can have hemobilia and intraabdominal bleeding in 0.5 % of cases.(9)
- Mortality rate among liver biopsy cases is about 0.009- 0.12%(10).
- Repeat biopsies are usually poorly tolerated by patients.

Hence due to these complications, it is challenged by recently increasing availability and validation of noninvasive methods for assessment of hepatic fibrosis.

b) Non-invasive:

There are various methods available for the assessment of liver fibrosis noninvasively can be classified into:

1. Serum markers
2. Imaging modalities.

1. Serum markers of fibrosis:

These are upcoming methods in this era of technology and modern methods in science for the assessment of fibrosis. As liver fibrosis is known to be reversible in its early stages these serum markers have made an increasing impact in its assessment. They are classified as direct and indirect methods. The direct markers measure the cause of the hepatocyte damage; whereas the indirect markers measure its consequences use routine laboratory tests. These are either used separately or as panels which are a combination of both to gives scores for the final assessment of liver fibrosis.(4).

There are various non-invasive methods to assess fibrosis used such as

a) Direct serum markers – Laminin, Hyaluronate, Metalloproteinases etc.

b) Indirect serum markers – AST/ALT ratio, APRI, Fibrosis probability index etc.

c) Serum panels (patented) – Hepascore R, Fibrotest, Fibroindex etc.

d) Imaging modalities – ARFI elastography, Transient elastography, MR Elastography etc.

In patients with hepatitis C of the chronic form hyaluronic acid which is a direct marker is the best single marker which is accurate and reliable for the assessment of severe degrees of fibrosis. Its use in other liver disease is also described and is valid. The direct markers assess the extracellular matrix turnover such as fibrogenesis and fibrinolysis(11). It has a high negative predictive value (NPV) of 98-100% and so it can be used on its own to assess liver fibrosis in the routine clinical settings (12). AST/ALT ratio which is a indirect marker for the assessment of liver fibrosis has shown good sensitivity (not very good specificity) to detect cirrhosis in patients with chronic hepatitis C with the ratio ≥ 1. It also has good positive predictive values (PPV) and NPV which is 73.7-100% and 46.7-53.2% respectively (12,13). Platelet count and prothrombin index are simple fibrosis markers which can be used for the assessment of liver fibrosis in routine clinical settings.

Various panels of test have been studied as the individual markers have fewer efficacies in picking up fibrosis and they are of good diagnostic value. Some of them are as follows:

The ratio of *ALT* to platelets which is better known as the APRI index and Fibrotest are the most extensively studied serum markers for the non-invasive assessment of liver fibrosis.(14). In the year 2007 a metaanalysis was done which showed that with a cut off value of 0.5 and 1 on APRI there was 81 % sensitivity and 50% specificity in diagnosing liver fibrosis and about 76% sensitivity and 71% specificity for the diagnosis of cirrhosis. (4,15) . This was termed as significant fibrosis (F≥2 on the METAVIR liver biopsy grading system. In another meta-analysis the AUROC of APRI for fibrosis ≥2, fibrosis of F3-F4 and cirrhosis the values were 0.77, 0.80 and 0.83.The sensitivity and specificity values for a APRI cut off value of 0.7 for significant fibrosis was found to be 77% and 72% and keeping the cut off value as 1 for cirrhosis the sensitivity and specificity were 61% and 64% respectively. (16). Therefore based on this data it is found that APRI has only moderate degree of accuracy in this group of chronic hepatitis C patients and cannot be used a sole routine diagnostic test and has to be combined with either another non-invasive test for precisely diagnosing liver fibrosis(4).

The Fibrotest comprising of five parameters namely haptoglobin, bilirubin, macroglobulin, apolipoprotein-A and gamma glutamyl transpeptidase is the most widely validated indirect serum marker panel which has been extensively used in

patients with CHC(17,18,) and also in patients with chronic hepatitis B, HCV/HIV co-infection and Non-alcoholic fatty liver disease (19)(20)(21).There was a review of about 9 studies which included around >1600 patients in which it was found that the area under the receiver operator curve for identifying cirrhosis and significant fibrosis (≥F2) was 0.9 and 0.81respectively showing that there was excellent discrimination between the two based on this Fibrotest. However, it was concluded that noninvasive tests cannot replace liver biopsy and can only be used in conjunction with the same(22).

Limitations

Although these tests are easy to perform, can be repeated periodically and highly applicable, they have certain limitations.

The various limitations are:

1. Inability to detect intermediate stages of fibrosis as compared to cirrhosis(23,24).

2. Patients with renal failure, liver failure, extrahepatic sites of fibrogenesis or patients in their postprandial state can show erroneous values with direct markers such as hyaluronic acid and so this test is not specific to the liver. (25)

3. There can be decrease in haptoglobin in patients with hemolysis, increase in bilirubin in patients with Gilbert's syndrome and increase in macroglobulin in inflammatory states which can lead wrong results of the Fibrotest.

4. Platelet count, International Normalised Ratio (INR) and transaminase levels can also differ between various laboratories which can also be a constant problem for their interpretation.

These panels can distinguish between significant versus no significant fibrosis but none of them reliably differentiate them into various stages and so they cannot be relied upon solely for clinical use in the diagnosis of fibrosis and cirrhosis.

2. Imaging Modalities:

Ultrasonography (USG), Colour Doppler, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are the imaging modalities commonly used in the assessment of changes of cirrhosis in liver such as liver surface, echotexture, volume redistribution or in assessing changes with splenoportal axis such as portal hypertension, collaterals, ascites etc. USG can non-invasively assess liver cirrhosis based on echotexture of the liver, surface of the liver; the latter being very sensitive and accurate for diagnosing liver cirrhosis(26).

But for the assessment of liver fibrosis before the patient presents with florid cirrhosis various imaging methods have been tried out non-invasively to assess the same; which if could pick up liver fibrosis can be used primarily just for assessing the intermediate stages of fibrosis and hence lead to improvement in treatment and prognosis in these group of patients. (27–30).

The technology uses expensive equipment and so it is difficult to be used in routine clinical practice; the cost factor being one of the most important major drawback.

Ultrasound Elastography:

Ultrasound-based elastography is rapid and noninvasively measures mean hepatic tissue stiffness. It is quick, inexpensive, reproducible, painless, and examines a large mass of liver tissue, thereby reducing sampling error.

There are two primary ultrasound-based elastography techniques used in clinical practice for evaluating liver stiffness; shear wave elastography (SWE) and strain elastography.

Both use mechanical excitation of the hepatic parenchyma with monitoring of the resulting tissue response(31). Fibrotic tissue is different from healthy tissue in the way it responds to excitation (shear waves propagate faster in fibrotic tissue, and fibrotic tissue when compressed shows less strain than healthy tissue).

SWE and strain elastography differ in the way the external mechanical excitation is applied and what quantity is measured.

Shear wave elastography is able to quantify elasticity and so it is dynamic in nature.

Strain elastography is semi quantitative and does not directly measure elasticity but rather determines elasticity relative to other structures(32). For both techniques translucent / colored elastograms are superimposed on conventional ultrasound B-mode images.

Shear wave elastography:

Shear waves can be generated from variety of sources, such as external pressure and vibration, normal physiologic cardiovascular motion, and acoustic radiation force impulse (ARFI).These shear waves can exist and can be propagated in any solid medium, including biologic tissue. Shear wave deformation occurs when any directional force is applied to any tissue. Shear wave propagation speed and the density of the material the shear wave is travelling through is what form the basis of liver stiffness measurements.

The stiffer the tissue the faster the wave propagates and so this shear wave speed is related to the liver parenchyma stiffness.

There are several methods for performing SWE, including transient elastography, point shear wave elastography, and two-dimensional (2D) Shear Wave Elastography.

The methods differ in how the shear wave is generated and in what measurements are taken.

In transient elastography (TE), the mechanical piston is mounted on a single element transducer which generates the shear waves. Skin is lightly pushed over an intercostal space, resulting in a shear wave that travels/courses through the liver. Measurements are generated along the direction of the ultrasound beam.

ARFI is the principle used in Point-SWE and 2D-SWE to generate shear waves. Focused acoustic radiation "push" pulses are used in ARFI elastography which

deforms the tissue and generates shear waves of low amplitude. These shear waves are tracked, and the distribution of displacement or its normalized value is displayed automatically on the USG screen. Shear wave speed measurements are taken either from one small area (usually 5 x 10 mm ROI in case of point-SWE) or from sequential measurement points (2D-SWE).

Strain elastography:

In this the tissue displacement is measured and converted to a strain image. The external mechanical excitation force is applied by compressing the liver (by transducer, cardiovascular pulsation, or respiratory motion). Fibrous tissue displaces less than normal parenchyma and strain images from fibrotic tissue will indicate less strain relative to normal tissue.

TRANSIENT ELASTOGRAPHY (Fibro Scan)(32)**:**

Echosens has developed this new modality and the principle is based on Hooke's law. There is external stress applied to a tissue which produces a strain response. This is a rapid, easily reproducible non-invasive technique which is used in assessing liver fibrosis. It also works by using the technique of shear wave velocity.

In this technique almost about 50 MHz wave is passed to the liver from a small vibrator which is attached to the end of an ultrasound probe. The vibrations are of low frequency however have mild amplitude and they are usually triggered from the one of the right intercostal spaces. The induced elastic shear wave courses through the liver

along the path of the generated waves and then this measurement is converted into liver stiffness and expressed as kilopascals.

The stiffer the tissue the faster the wave propagation occurs giving higher values of liver stiffness in kilopascals. Pulse wave ultrasound acquisition is used to follow the shear wave propagation and to measure its speed.

$E = 3qV^2$ is the formula used where V is the shear wave speed and q is the material density (constant for tissues of interest). Liver stiffness measurement using transient elastography is usually a large representative area of the liver (~ 10 mm wide and 40 mm long). It has a M-probe for measuring liver stiffness in normal built patients at a depth of 2.5 cm upto 6.5 cm from the skin surface. For paediatric and obese age group the S and XL probes are now newly available for accurate measurements of liver stiffness. In this technique liver stiffness is measured in kilopascals (kPa) with a normal range of 2.5 kPa to 75kPa. The volume of liver tissue it measures is a cylindrical volume and is much bigger than a normal liver biopsy sample; hence is more representative of the hepatic parenchyma when results are compared. It cannot be performed by the subcostal approach or via the left lobe of liver and only a few intercostal spaces on the right can be used. The position of the patient, body habitus, subcutaneous fat, the intercostal space from which this is performed, presence of ascites all play an important role in the interpretation of results and also lead to signficant inter and intraobserver variability.

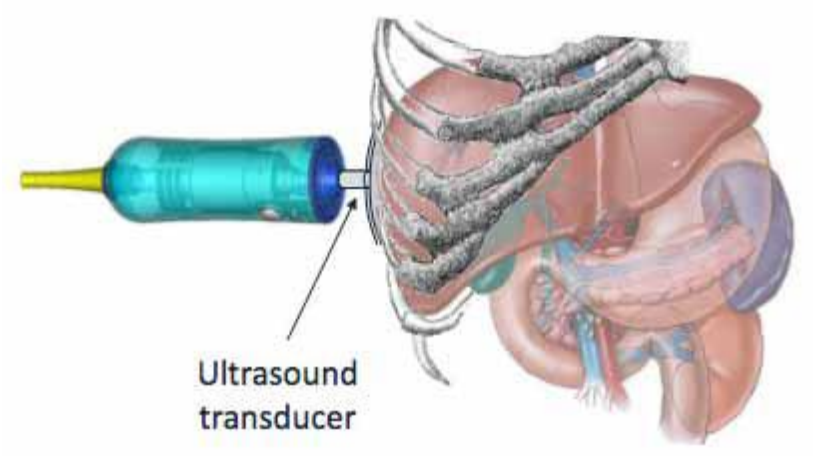


Diagram 3: Transient Elastography for the Measurement of Liver Fibrosis.(33)

Transient elastography is a good beside procedure which is painless and easily reproducible; the results of which are immediately available. It also has good inter and intraobserver agreement. Therefore, this is a good and upcoming technique for the assessment of liver fibrosis.

Limitations: TE does not give a grey scale image of the tissue under examination but provides only an elasticity value from the place of measurement. In patients with obesity, ascites and its limited depth of examination ranging from 2.5 to 6.5 cm limits its use in the general population.

Validity of TE is based on two important parameters: (1) the success rate (which is the ratio between the number of successful measurements to that of the total number of measurements) ~ 60%; and (2) the interquartile range (IQR), which is the variability between two valid measurements, which should not exceed 30% of the median

value(34). There can be unreliable measurements in patients with high body mass index (BMI)> 30 kg/m^2, in women, and in patients with diabetes mellitus.

There can be about 5% loss in number of acquisitions in patients with obesity.

The values of TE should always be interpreted by an expert clinician with knowledge about all the various know parameters.

The values of TE range from 2.5 to 75 kPa. There are available cut off values for depending on the underlying liver disease for diagnosing significant fibrosis (F≥2) and cirrhosis (F4). The cut offs used in common clinical setting is >7 kPa for significant fibrosis (F2 to F4) and >11 to 14 kPa for cirrhosis. The contiguous stages of liver fibrosis however cannot be differentiated on Transient elastography.

In a study done by Fraquelli et al.(35) on the role of TE in patients with β-thalassemia major patients; it was observed that between liver stiffness and fibrosis stage a significant positive correlation (r = 0.73, P = 0.003) was found in all patients who underwent liver biopsy. A sensitivity of 60% and a specificity of 89% was found in patients with severe fibrosis and a sensitivity of 100% and a specificity of 92% in those with of cirrhosis.

Erhardt et al.(36) in their study on diagnosis of liver cirrhosis using transient elastography in comparison to liver biopsy scores showed that the areas under the receiver operating curve (AUROC) were 0.91 for ≥ F3 fibrosis (95 % CI: 0.85 - 0.96)

and 0.94 for cirrhosis (95 % CI: 0.90 - 0.98) showing that results of transient elastography correlated positively with the histological score of liver fibrosis (r = 0.8; 95 % CI: 0.72 - 0.85; p < 0.001). Keeping 13 kPa as cut off for detection of liver cirrhosis it has a sensitivity, specificity, PPV and NPV of 90 %, 82 %, 71 % and 95 % respectively.

Image courtesy: UpToDate 2015-Transient elastography showing the measurement of liver stiffness in kilopascals (kPa) along the left side of the screen (37).

SHEAR WAVE ELASTOGRAPHY — Shear wave speed measurement using acoustic radiation force impulse (ARFI) imaging is an alternative to transient elastography. It has an advantage of combining conventional ultrasound with liver stiffness measurements.

In addition, methods that use ARFI can obtain liver stiffness values in patients with ascites and may be less influenced by obesity than transient elastography.

It is of two types: Point shear wave elastography and 2D shear wave elastography.

Point-shear wave elastography — Point-SWE simultaneously displays the shear wave speed and conventional ultrasound images. The same ultrasound transducer is used to generate the shear waves for imaging the wave propagation. In a meta-analysis done by Bota et al. the sensitivity and specificity of point-SWE for the diagnosis of significant (F≥2) fibrosis is approximately 75% and 83%, and for diagnosing cirrhosis (F4) is approximately 87% and 87% respectively(38).

Image Courtesy: UpToDate 2015: Point-shear wave elastography using acoustic radiation force impulse in healthy liver parenchyma (A) and complete cirrhosis (B). The shear wave speed and the depth of the region of interest (rectangular box) are shown on the right side of the image (39).

However, similar to transient elastography, point-SWE also cannot differentiate between various stages of liver fibrosis.

In a pooled meta-analysis done by Friedrich-Rust et al.(40) on the performance of Acoustic Radiation Force Impulse elastography for the staging of liver fibrosis in patients with chronic liver disease the optimal cut-off values for the diagnosis of fibrosis with their respective sensitivities and specificities were:

- F≥2: 1.34 m/s, sensitivity -79%, specificity - 85%
- F≥3: 1.55 m/s, sensitivity - 86%, specificity - 86%
- F4: 1.80 m/s, sensitivity - 92%, specificity - 86%

Similar to TE a right intercostal approach is preferred when performing point-SWE. However, unlike transient elastography, other approaches may be used. Minimal transducer pressure and a short breath hold in the mid-respiratory position (avoiding breath hold in deep inspiration) are used to improve the reproducibility of measurements. Liver stiffness is reported as an average value within a region of interest (point measurement). The values are reported as either shear wave speed (m/s) or converted to kPa (elastic modulus). The shear wave speed is directly proportional to the square root of the tissue elasticity.

Limitations of point-shear wave elastography:

Many of the findings are nonspecific and obtaining the values and interpreting them is highly operator dependent.

Point-SWE has narrow range of values (0.5 to 4.4 m/s), which may limit the ability to define optimal cutoff values.

Another limitation is that patients who have elevated aminotransferase levels have led to overestimation of hepatic fibrosis.

Two-dimensional shear wave elastography

2D shear wave elastography is also known as Aixplorer, SuperSonic Imagine (SSI) and has been introduced as a 2Das well as a 3D-technique. 3D technique has been used in studies on the breast however 2D has been tried upon the liver.

Technique: 2D-SWE examination is performed with conventional ultrasound guidance using a conventional ultrasound probe. The right intercostal approach is preferred, similar to the transient elastography examination technique; but other approaches may also be used. The patient lies down with maximum right arm abduction in the supine position which makes the right hypochondrium accessible. Pressure is applied to the transducer to enlarge the intercostal space, decrease the thickness of the subcutaneous fatty layer, and ensure optimal contact between the probe and the skin. The probe must be placed parallel to the intercostal window to avoid shadowing from the ribs. A breath suspension in the mid-respiratory phase

(avoiding breath hold in deep inspiration) improves the reproducibility of measurements. The shear wave elastography map is placed in an area that is free of blood vessels with a uniform image on B-mode, at least 2 cm below the liver capsule. The region of interest is placed in the central area of the shear wave elastography map, over an area of relative homogeneous elasticity.

This technique combines high frame rate USG imaging (~ 5000 frames/sec) along with radiation force induced in the tissue by focused beams. This in turn is able to catch the propagation of resulting shear waves on real time imaging.

A dedicated time-of-flight estimation technique is used to recover the shear waves which in turn enables for 2-D mapping of elasticity quantitatively. On the 2D image; 3 supersonic shear wave imaging sequences are applied successively to the left, middle and right parts of the ultrasound image, which in turn gives three elasticity measurements. The combination of these values gives the liver moduli which are usually taken from healthy volunteers from in vivo data, which were consistent with those reported in the literature (4 to 7.5 kPa). This technique is also fast, can be easily repeated and has good reproducibility. It is reported to have good reproducibility with high intra- and inter-observer agreement.

Clinical uses and application:

Ferraioli et al. (41) in his study on 121 patients with chronic hepatitis C assessed the accuracy of two techniques; 2D shear wave elastography(2D-SWE) versus transient

elastography(TE) using liver biopsy as the reference standard, and found that the area under the receiver operator curve (AUROC) was 0.92 for 2D-SWE and 0.84 for TE (*P* = 0.002)for F0-1 versus F2-4 fibrosis; 0.98 for 2D-SWE and 0.96 for TE (*P* = 0.14)for F0-2 versus F3-4 fibrosis and 0.98 for 2D-SWE and 0.96 for TE (*P* = 0.48) for F0-3 versus F4 respectively. Values increased sequentially along with the degree of liver fibrosis both with 2D-SWE and TE. It was found that for fibrosis (≥ F2) 2D-SWE was more accurate than TE.

Image courtesy: 2D-SWE and supersonic shear imaging for the evaluation of biopsy proven liver fibrosis and cirrhosis. A: F0; B: F1; C: F2; D: F3; E: F4 = cirrhosis(32).

2D-SWE can be used in patients with ascites. Studies suggest that the sensitivity for diagnosing significant fibrosis (F≥2) is 77 to 83 percent, with a specificity of 82 to 83 percent. The sensitivity for diagnosing cirrhosis is 81 to 85 percent, with a specificity of 61 to 83 percent.

Efficacy of 2D-shear wave elastography:

STRAIN ELASTOGRAPHY(41–43):

Strain elastography (or real-time tissue elastography, quasistatic imaging, strain imaging,) measures the strain response of tissue to stress, either by as manual compression or by cardiovascular pulsation, and is a relative measurement of tissue elasticity. It has been successfully used to evaluate lesions in the breast, thyroid, pancreas and lymph nodes, but its role in the assessment of liver fibrosis is unclear because experience with this technique is limited.

Also, there is a lack of standardization for this technique and is nonquantitative in nature.

Because the liver is deeply located, compression applied at the body surface may not be readily transmitted, making it difficult to elicit strain from the body surface. Therefore, strain induced by either cardiac pulsation or respiration is typically used for evaluation of liver fibrosis.. The distribution of strain values can be displayed as a histogram and measurements such as mean strain, standard deviation from the mean, and percentage of blue pixels can be made and have been shown to correlate with increasing degrees of liver fibrosis.

Technique:

Harder tissues are less compressible than soft tissues; this forms the basis of strain elastography. When a compression is applied with a probe in a subtle manner there is a relative degree of tissue strain but no direct physical elasticity. The results of strain elastography are displayed as colours ranging from red to blue (indicating softer to harder tissue). This is calculated by the strain response of each tissue to stress (which is relative tissue elasticity). When a defined region of interest is to be measured quantitatively semi-quantitative elastography techniques can also be used. Both the B-mode image and corresponding tissue elasticity image could be displayed simultaneous in real time if a high speed algorithm is used hence capturing both accurately.

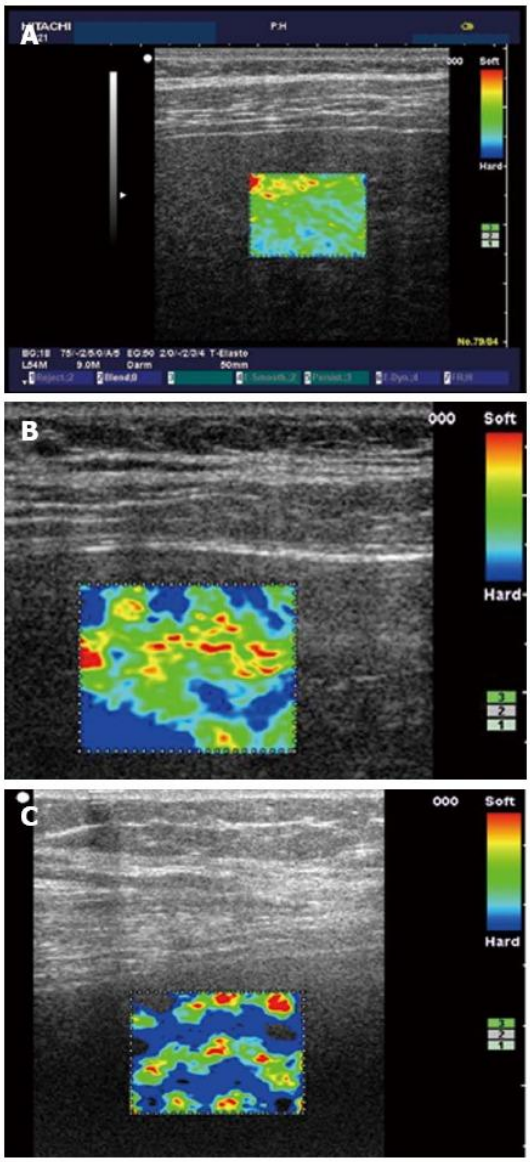


Image courtesy: Strain elastography for the evaluation of biopsy proven liver fibrosis and cirrhosis. A: F0; B: F2; C: F4 = cirrhosis.(32)

Because manual compression would carry a chance of false interpretation of the strain value in terms of the scoring of elasticity as well as the image; a recent improvement in this technique by the Hitachi medical systems has been developed which requires no extra external stress on the tissue. The heart and abdominal aorta would sufficiently

provide rhythmic pulsations to give the required amount of distortion required to generate a strain into the liver tissue which would provide adequate information about the elasticity of the liver. Therefore the compression provided by the performer would no longer be a problem for the assessment.

Frederich-Rust et al. in the year 2007 (44) found the clinical application of SE in the liver. The diagnostic accuracy using the Matlab computer program for F2, F3 and F4 were 0.75, 0.73 and 0.69, respectively which automatically generated a colour code image to show the strain values and also automated elasticity scores.

Again in the year 2009 the same group compared strain elastography and transient elastography along with comparing other serum fibrosis markers and found that transient elastography was superior to strain elastography. However after the development of superior techniques by the Hitachi medical systems, there were good results available in various studies.

Morikawa et al (45) in their study on comparison of the diagnostic accuracy of strain elastography versus transient elastography for diagnosing liver fibrosis found that SE can be used as a routine imaging tool for evaluation of liver fibrosis. In this technique the pixel data in the range of interest was transferred into a histogram and a binary image. This was then quantified using a particular devised system where it was found that the mean value on the histogram and the percentage of hard tissue directly represented the elasticity of the liver tissue.

Further studies in this field of strain elastography are required for the evaluation of liver fibrosis to establish an accurate method of assessment and analysis of the values of this technique.

Advantages:-

Just like other non-invasive techniques this can also be used in a large region of interest and it can measure the patchy and hard nature of liver fibrosis as the disease progresses from a uniformly normal and soft looking liver.

Efficacy of strain elastography — Strain elastography has been studied in the liver primarily for assessing liver fibrosis and investigating liver tumors. Several different elasticity score methods have been published. The sensitivity and specificity of strain elastography in a meta-analysis of about 15 studies with > 1600 patients, for significant fibrosis (F≥2), severe fibrosis (F≥3), and cirrhosis (F4) were 79, 82, 74 percent and 76, 81, 84 percent respectively. However, the authors noted that the sensitivity and specificity may have been overestimated because there were signs of publication bias.

In a study that compared strain elastography with point-shear wave elastography (SWE) and transient elastography, there was no statistically significant difference among the three techniques for the diagnosis of cirrhosis; however point-SWE and transient elastography were better than strain elastography for the prediction of significant fibrosis.

Acoustic Radiation Force Impulse (ARFI) Elastography:

- Principle and protocol for ARFI elastography of liver
- How is ARFI done?
- Diagnostic accuracy of ARFI in staging liver fibrosis
- Reproducibility of ARFI elastography
- Factors influencing ARFI elastography measurements
- Limitations of the ARFI study in children
- ARFI elastography vs Transient elastography (TE) and its advantages over Transient Elastography.

ACOUSTIC RADIATION FORCE IMPULSE ELASTOGRAPHY (ARFI)(46–49)**:**

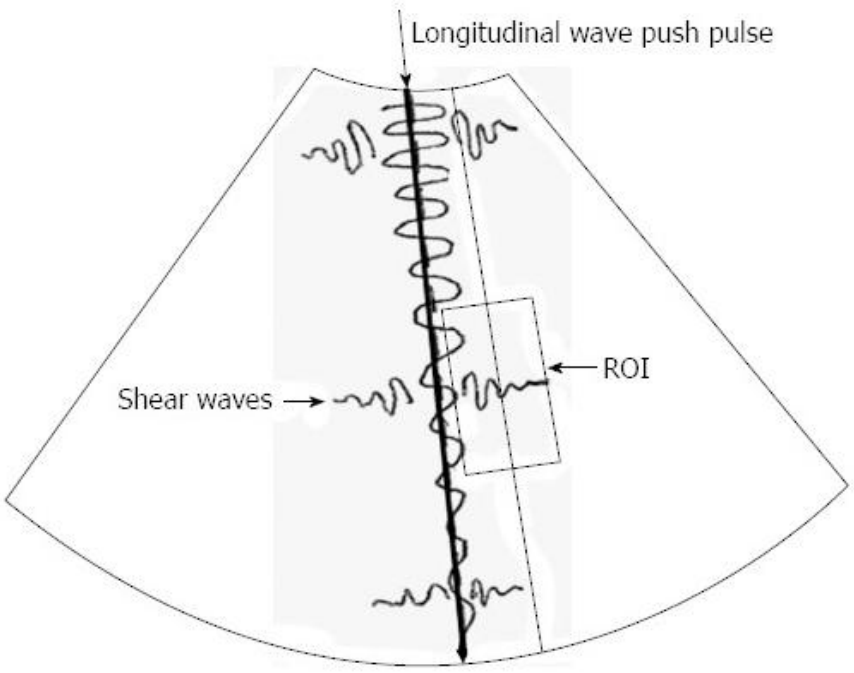
(i). PRINCIPLE:

It is a novel, cost effective and promising ultrasound-based technique that allows the assessment of the tissue stiffness by evaluating wave propagation speed. It is implemented in the conventional ultrasound machine. Localized displacements are generated in a selected region of interest by short-duration acoustic radiation force (less than 1 ms) which does not require any external compression which in turn reduces the operator dependency. The generated wave gives either the qualitative or quantitative (shear wave velocity values in m/sec) responses.

ARFI has similar accuracy in assessment of liver fibrosis and cirrhosis when compared to transient elastography and it also has certain advantages in respect to transient elastography in terms of using the same ultrasound equipment, better display of the anatomical structures and greater success in obtaining measurements in almost every patient.

Acoustic radiation force impulse (ARFI) elastography is acoustic radiation force based imaging method combined with conventional B mode ultrasound developed by (Acuson S 2000, SEIMENS Medical Solutions). The 'Virtual Touch Tissue Quantification' (VTQ) provided by SEIMENS quantifies the tissue stiffness by shear wave velocity of acoustic radiation force impulse displacement within the human tissues. It is represented as m/sec and is directly proportional to the square root of the tissue elasticity.

To obtain a baseline signal, an initial ultrasonic pulse at intensity level used for normal diagnostic imaging is transmitted by the transducer. Followed by this a short duration (0.3 s) high intensity acoustic 'push pulse' is applied by the same transducer, followed by multiple diagnostic pulses. These diagnostic pulses track the displacement of the tissues caused by the push pulse. Using the conventional B-mode imaging the response of the tissues to the radiation force is assessed and represented as shear wave velocity measurements as m/ sec.



Acoustic radiation force impulse virtual touch tissue quantification technical scheme. ROI: Region of interest.

Diagram 4: Representative image showing the mechanism of ARFI elastography.

(ii). PROTOCOL FOR ARFI ELASTOGRAPHY OF LIVER:

ARFI elastography Virtual Touch Tissue Quantification imaging is normally performed with curved array 4 MHz, B-mode ultrasound transducer. The anatomic region of interest is assessed using a 'region of interest' (ROI) box which measures ~ 10 X 5 mm and measures the shear wave velocity simultaneously with real time B-mode USG imaging. The patient lies with maximal right arm abduction in the supine position. The right lobe of the liver is assessed through the intercostal approach. The other approach is the abdominal approach; the intercostal approach has been found to be superior to the abdominal approach.

(iii). REGION OF INTEREST (ROI) PLACEMENT IN THE LIVER ARFI ELASTOGRAPHY:

Goertz et al, in their study done on 57 patients found that the best ARFI measurements were using the intercostal approach to segments VII/ VIII of the liver(50) with lowest rate of inaccurate measurements. In another study done by Bota et al.(51) among 83 patients in segments V and VIII of liver, there was no significant statistical difference between the mean liver stiffness between the two segments. It was found that the correlation between ARFI and fibrosis was similar in the segments 5 and 8 of liver ($r=0.836$ vs. $r=0.784$) ($p=0.33$). There was no difference between the mean liver stiffness values between right and left lobe of the liver (2.06 ± 0.1 vs.2.08 ± 0.98 m/s, $p=0.89$).

The analysis of the right lobe was found to produce consistent values. The left lobe measurements can have wrong values, due to the interference of the shear wave by the cardiac pulsations. The ROI is placed 2-3 cm from the surface of the liver for accurate assessment, not exceeding a depth of 5.5 cm(52). There is no definite consensus on the depth of the ROI from the liver surface.

Short duration acoustic pulses with a fixed transmitted frequency of 2.6 MHz is used to mechanically excite and cause localized displacement of the tissues within the ROI. The tissue displacements create a shear wave propagation which is away from the

region of excitation. The ultrasound beam tracking laterally to the single push beam is estimated as the maximum displacement. The propagation velocity within the tissue is proportional to the square root of tissue elasticity. The result is expressed in meters per second (range, 0.5–4.4 m/s with ± 20 % accuracy over the range).

While taking the ARFI measurements, the patient is asked to stop breathing momentarily. Minimum of 10 measurements are taken from the liver and the median value is calculated. A good assessment using ARFI should include IQR less than 30 % and success rate greater than 60% (53).

(iv). DIAGNOSTIC ACCURACY OF ARFI ELASTOGRAPHY IN STAGING LIVER FIBROSIS:

In a study done by Bota et al(54) on patients with chronic hepatitis C, on whom ARFI was performed and subsequently liver biopsy was done. 10 reliable measurements were done and a median value was obtained with a success rate ≥ 60% and an interquartile range interval < 30%. The cut off values were taken from their own metaanalysis and kept at 1.35 m/sec for F≥2 and 1.87 m/sec for F=4.It was found that 58 patients had LS values <1.35 m/s; out of which 75.8% had F ≥ 2 in biopsy. Among the 59 patients who had with LS values ≥ 1.35 m/s, only 6.8% had F0 or F1 in LB. Also, 88 patients (75.3%) had LS values <1.87 m/s; out of which only 2.2 % had F4 in LB and out of the 29 patients (24.7%) with LS values ≥ 1.87 m/s, 41.3% had F4 in liver biopsy. It was concluded that ARFI elastography had a good PPV (93.2%) for

predicting significant fibrosis and excellent NPV (97.8%) for excluding compensated liver cirrhosis.

Nierhoff et al.(55) , performed systematic literature review of 36 articles and approximately 3,951 patients evaluating the diagnostic accuracy of ARFI in staging liver fibrosis were the inclusion criteria were all the studies which evaluated the efficacy of ARFI elastography of liver with liver biopsy as reference standard. The METAVIR scoring system for liver fibrosis staging on biopsy was used. AUROC for fibrosis stage F > 2, F > 3, F = 4 according to METAVIR or comparable liver fibrosis scoring system, all the studies which studied the sensitivity, specificity, positive predictive value, negative predictive value according to the various ARFI shear wave velocity cut off values for various stage of liver fibrosis on biopsy were taken into account.

If the AUROC is 1 then a diagnostic tool is defined as perfect, if it is greater tha 0.9 then it is termed as excellent and is called good when it is greater than 0.8. The mean diagnostic accuracy of ARFI expressed as the AUROC was 0.84, 0.89 and 0.91 for significant fibrosis (F≥2), severe fibrosis (F≥3) and cirrhosis (F=4) respectively.

Hence it was proven that ARFI elastography of the liver has good diagnostic accuracy for detecting significant liver fibrosis (F > 2, F > 3) and has excellent accuracy for liver cirrhosis (F=4).

(v). REPRODUCIBILITY OF ARFI:

Since ARFI is real time ultrasound based quantitative measurement of tissue stiffness, interobserver variability has been studied and it found that there is good agreement in

this test. According to Friedich Rust et al, there was 87% agreement between two observers regarding ARFI derived elastography stages(56).

(vi). ARFI ELASTOGRAPHY SHEAR WAVE VELOCITY (SWV) VALUES OF LIVER IN NORMAL HEALTHY SUBJECTS AND ITS CORRELATION WITH DIFFERENT STAGES OF FIBROSIS ON LIVER BIOPSY:

Several studies have been done among healthy volunteers to determine the normal liver stiffness values by ARFI elastography.

Study done by Popescu et al(57) found the mean shear wave velocity of liver as 1.15 ± 0.21 m/s. Various other studies mention the mean shear velocity of liver cut-offs among healthy volunteers, as 1.08 ± 0.15 m/s.

Kirches at al. in their study found the normal values and the shear wave velocity values of significant fibrosis and cirrhosis of liver using ARFI elastography with transient elastography as the reference standard. The study enrolled 666 patients out of which 68 patients underwent liver biopsy. In this study they have found that the there was significant success rate of ARFI of liver as compared to FS [604/606 (99.7%) vs 482/606 (79.5%), $P < 0.001$]. There was significant correlation between ARFI-SWV and FibroScan liver stiffness (FS-LS) ($r = 0.920$, $P < 0.001$). As the stage of fibrosis increased, there was significant increase in the ARFI-SWV. For patients with no significant liver fibrosis, ARFI-SWV was found to be 1.09 ± 0.13 m/s and with FS-LS

< 7.6 kPa); for patients with significant liver fibrosis as 1.46 ± 0.27 m/s (FS-LS ≤ 13.0 kPa); and for patients with liver cirrhosis as 2.55 ± 0.77 m/s (FS-LS > 13.0 kPa).

Thus, according to Kirches at al. ARFI-SWV cut-off values of liver with no significant fibrosis was found to be (1.29 m/s; sensitivity, 91.4% and specificity 92.6%) and for liver cirrhosis (1.60 m/s; sensitivity, 92.3% and specificity 96.5%). The optimal cut-off ARFI shear wave velocity measurement for predicting liver fibrosis (F ≥ 2) was 1.32 m/s with a sensitivity and specificity of 87.0% and 80.0% respectively) and for cirrhosis (F4) 1.62 m/s (with a sensitivity and specificity of 100% and 85.7% respectively), in the patients who underwent liver biopsy. There was excellent inter- and intraobserver reproducibility for ARFI elastography also seen in this study(58).

(vii). FACTORS INFLUENCING ARFI ELASTOGRAPHY MEASUREMENTS:

The most important factor affecting the ARFI elastography score is the liver stiffness which is a surrogate marker for the extent of liver fibrosis. However, they are few factors which have been noted which affect the shear wave velocity measurements.

According to Rifai et al. the presence of liver inflammation causes increased shear wave velocity as opposed to those with no significant inflammation. They also found a positive correlation between the liver and the spleen size and the ARFI measurement (21).

Takahashi et al. reported a positive correlation with increased ARFI measurements and elevated serum aspartate aminotransferase, alanine aminotransferase levels and liver pathological inflammation (22).

Pathological liver steatosis has been found to be a significant factor which can affect liver ARFI measurements. Yoneda et al. suggested that ARFI measurements in liver steatosis are slower than normal individuals. Near normal ARFI values were seen in patients with NAFLD and mild liver fibrosis. It was suggested that the presence of steatosis made the liver softer and thus causing low ARFI measurements (23).

Patients with BMI greater than 40 kg/ m2 require XL ultrasound probe for ARFI measurements. However the standard cut-off values in this subset of patients has not yet been standardised.

(viii). LIMITATIONS AND CONTRAINDICATIONS OF ARFI ELASTOGRAPHY:

There are no significant contraindications or limitation to ARFI elastography evaluation of the liver. The limitations of obesity, narrow intercostal space and ascites which were encountered by transient elastography, which do not apply for ARFI elastography in the general population; however in children narrow intercostal space is a limitation.

Also breath holding during the ARFI scan is difficult in children leading to spurious values in these groups of patients.

(ix). ARFI ELASTOGRAPHY OF LIVER IN CORRELATION WITH TRANSIENT ELATOGRAPHY:

Kirches et al, performed TE measurements of liver in six hundred and sixty six patients and compared them to ARFI and sixty eight patients and compared them with the fibrosis stage on liver biopsy. The overall success rate for ARFI was 93.3% ± 9.87% ($P < 0.001$) as compared to TE which was 77.8% ± 28.5%. The success rate of liver stiffness measurement of 100% was observed in 373 patients (61.6%, $P < 0.001$) by ARFI than compared to 262 (43.2%) patients by transient elastography.

There was significant correlation between TE and ARFI ($p< 0.001$). Mean ARFI shear wave velocity measurements significantly increased with the stage of fibrosis. For patients with no significant fibrosis, the median ARFI value was 1.09 ± 0.13 m/s and (FS-LS < 7.6 kPa), for patients with significant fibrosis the median ARFI value was 1.44 ± 0.26 m/s (7.6 < FS-LS ≤ 13.0 kPa); and 2.55± 0.77 m/s for patients with liver cirrhosis (13.0 < FS-LS). Cut-off values for patients with no significant fibrosis and patients with liver cirrhosis were taken in order to obtain the maximum sensitivity and specificity values. With ARFI shear wave velocity of 1.29 m/s a sensitivity of 91.4% and specificity of 92.6% was found for patients with a value of < 7.6 kPa on transient elastography and a cut off value of 1.60 m/s was found for patients with > 13.0 kPa on TE with a sensitivity of 92.3% and specificity of 96.5%. For significant fibrosis and cirrhosis both the methods had high diagnostic accuracy.

(x). ADVANTAGES OF ARFI ELASTOGRAPHY OVER TRANSIENT ELASTOGRAPHY:

ARFI as compared with TE has the advantage that it is integrated into a conventional ultrasound system. This enables measuring elastography scores of the liver and at the same time allows screening of focal liver lesion in patients with suspected chronic liver disease with the same ultrasound machine and the probe. In addition, the site of measurement ARFI can be visualised with B-mode ultrasound which allows more exact measurement of liver tissue elasticity by excluding small non-parenchymatous areas like the gall bladder, portal and biliary radicals within the ROI measurement site.

ARFI can be performed in both the lobes of the liver, which may enable a better overall estimation and distribution of liver fibrosis. Multiple biopsies would be required from different segments of the liver to assess accurate comparison with ARFI imaging. An advantage of TE over ARFI elastography is the larger measurement area of 4 cm in length, as compared to ROI of 1 cm in ARFI. This shortcoming is overcome by as ARFI elastography includes multiple measurements from the liver. The success rate of ARFI is better than transient elastography.

The table given below gives a comparison between various non-invasive methods in the assessment of liver fibrosis and the advantages and disadvantages of each test.

(xi). ARFI IN CHILDREN AND ITS USEFULLNESS:

In a prospective study done by Noreugas et al(59) among children with chronic liver disease; ARFI elastography was compared with that of and/or before liver transplantation. Also children with no liver disease were taken as controls for this study. Among the controls the mean shear velocity was found to be 1.19 m/sec and among the children with liver disease it was found to be 1.70 m/s. The AUROC were 0.834, 0.818 and 0.983 for fibrosis stage of ≥ F1, ≥F2 and F4 respectively. As this test showed good correlation it was concluded that ARFI can be a good method for non-invasively assessing liver fibrosis and could be an alternate technique to liver biopsy as well.

Transient elastography has been studied in children with Thalassemia major and it is found that Di Marco et al.(60) and compared to METAVIR histological grades on 56 patients and it was found that liver stiffness increased proportionally to liver fibrosis staging ($r = 0.70$; $P > 0.001$).The AUROC for prediction of cirrhosis was 0.997 with cut-off of 13 kPa with 100% sensitivity (95% CI: 69.0-100.0) and 95% specificity (95% CI: 84.2-99.3). Therefore, Transient elastography is found s a good and reliable non-invasive method for assessment of advanced liver fibrosis in thalassaemia major patients.

There are no studies till date which gives the importance of ARFI in children with thalassemia in comparison to the histopathological grades on liver biopsy.

Table below shows the various noninvasive methods to evaluate liver fibrosis along with its usefulness and drawbacks.

	Advantages	**Disadvantages**
Transient Elastography	High accuracy, reproducibility and easy to learn	* Technical requirements * Limited recognition of intermediate stages of fibrosis * Cannot be performed in obese patients and those with ascites; false positivity in patients with hepatitis, cholestasis and heart failure
Point SWE	* High accuracy, reproducibility and easy to learn with conventional USG images also obtained * Can be done in obese patients and in patients with ascites	* Technical requirements * Intermediate stages of fibrosis not well distinguished * Narrow range of values
2D-SWE	* High accuracy, reproducibility and easy to learn with conventional USG images * Can be performed in obese patients and patients with ascites	* Technical requirements * Limited recognition of intermediate stages of fibrosis

	* Larger area of measurement than other USG techniques	
MR Elastography	* High accuracy and reproducibility * Conventional MRI images also obtained	* Technical requirements * High-cost and time consuming * Intermediate stages of fibrosis not well recognised * Not applicable in cases of iron deposition
Serum Biomarkers	*Availability * Reproducibility	* Non-specific(Hyperbilirubinemia, hemolysis and inflammation * High cost * Limited recognition of intermediate stages of fibrosis * Results not immediately available

MRI Elastography (MRE) (61):

It is usually performed on a 1.5 T MRI machine, using a transmit/receive body coil. There is an acoustic driver device placed on the anterior body wall which releases low amplitude mechanical waves of about 60 Hz.

The acquired images of propagating shear waves are processed and MR elastograms are displayed which quantitatively measures the shear stiffness with the help of previously described local frequency estimation inversion algorithm technique.

Yin et al. in their study on 35 normal volunteers and 50 patients with chronic liver disease found that liver stiffness increased systematically with fibrosis stage. Area under the receiver operator curve with a cut of 2.93 kPa showed a 98% sensitivity and 99% specificity for diagnosing all the grades of fibrosis. This study also showed that patients with moderate and severe fibrosis could be differentiated from the milder ones on MR elastography with a sensitivity of 86% and specificity of 85%.

To assess the potential of fat infiltration to have an effect on the stiffness measurement; liver fat versus water ratios were also calculated and it was found that there was no effect of steatosis on the liver stiffness measurements. It was also found that obesity and ascites do not hinder the assessment of liver fibrosis in this technique.

In a metaanalysis done by Singh et al.(62) which analyzed data from 12 retrospective studies, comprising 697 patients it was found that the AUROC of MRE for diagnosis of any (≥ stage 1), significant (≥ stage 2), or advanced fibrosis (≥ stage 3), and cirrhosis was 0.84, 0.88, 0.93, and 0.92, respectively, suggesting excellent discriminative ability for the detection of advanced fibrosis and cirrhosis, and good discriminative ability for the detection of any and significant fibrosis. The corresponding MRE liver stiffness cut-off values were 3.45, 3.66, 4.11, and 4.71 kPa, respectively. Based on the estimates of sensitivity and specificity, it was estimated that

a high positive and negative likelihood ratio is found particularly for the detection of advanced fibrosis (sensitivity and specificity of 85% and 85% respectively) and cirrhosis (sensitivity and specificity of 91% and 81% respectively).

So, MRE is also a promising technique for the non-invasive assessment of liver fibrosis which can distinguish various stages of liver fibrosis and hence holds a good chance of replacing invasive methods like liver biopsy in future.

Assessment of Serum Ferritin levels in Thalassemia and its relevance:

Ferritin is a storage form of iron which store the iron safely and in a non-toxic manner; for use when needed. Serum ferritin levels are usually done as part of the iron workup for patients with anemia. They are directly proportional to the total amount of stored iron in the body. It can be erroneously elevated in the form of an inflammatory acute phase protein in patients with anemia of chronic disease; where it is not a marker for iron overload.

The normal range of serum Ferritin for various age groups is as follows:

Men and Women > 50years(CMC)	20-320 ng/mL
Women < 50 years (CMC)	10-290 ng/mL
Children (above 6months to 15 years)	7-140 ng/mL
Infants (upto 5 months)	50-200 ng/mL

If ferritin is high, then there can be excess iron overload in the body, an acute inflammatory reaction in which ferritin is mobilized without iron excess or the patient is suffering from an acute infection where the ferritin levels may be high without any cause of iron overload. In patients with hemochromatosis and hemosiderosis it is used as a marker for iron overload disorders. Infections and hepatic diseases also can cause false increase in the level of serum ferritin.

In patients with Thalassemia major Ferritin levels are high due to transfusional iron overload.

There are various studies done to predict various risks of heart failure in patients with Thalassemia major which is a major cause of mortality in these patients by estimating serum Ferritin levels in comparison to liver T2* imaging and cardiac T2* imaging.

In a study done by Eghbali et al. (63) on assessing serum Ferritin levels and MRI T2* imaging of heart and liver; no significant association (p=0.361, r=-0.120) was found between the cardiac T2* imaging and serum ferritin levels; however there was a significant correlation between serum Ferritin and liver T2* Imaging (p=0.021, r=-0.297).There was increase in the value of Serum Ferritin in comparison to the worsening hepatic T2 relaxation times.

It is a good method of iron overload estimation along with MRI T2* imaging; however its sole use is questionable and can be used with moderate sensitivity in areas where MRI T2* imaging is not available.

In another study done by Majd et al.(64) Serum Ferritin levels had a statistically significant correlation with the Liver Iron concentration with a P value of < 0.001).

MATERIALS AND METHODS:

STUDY DESIGN: Test of diagnostic accuracy. Prospective study approved by the Institutional Review Board (IRB). IRB study number – 8659 and Fund no. 22Y330

SETTING: Christian Medical College (CMC) Vellore in Tamil Nadu is a tertiary care hospital established in 1900. It is a 2700 bedded hospital with multiple specialities and super specialities. The no of outpatients and inpatient handled on an annual basis is around 2 million and 1, 30,000 respectively.

The department of Radiology was established in the year 1936 with conventional radiography and moved into digitalization in the year 2000 with introduction of the Picture Archiving and Communication Systems (PACS).

The department is staffed by 70 radiologists and about 120 radiographers.

The radiological procedures performed on a routine basis are radiographs, IVU, Barium, USG, Doppler, Mammography, CT and MRI.

METHODOLOGY:

1. SAMPLE SIZE:

The total sample size calculated was 60. However due to a set number of patients who undergo bone marrow transplantation; which is about 3-4 cases/months; 54 cases were recruited. There was one insufficient sample on liver biopsy and so analysis will be done on 53 cases. However, study will be ongoing till a total of 60 cases are done.

2. INCLUSION CRITERIA FOR THE STUDY:

All the patients in pediatric age group (age 2- 20 years) with Thalassemia major who are planned for a liver biopsy prior to the Bone Marrow Transplantation (BMT).

3. EXCLUSION CRITERIA FOR THE STUDY:

1. Those patients who could not undergo liver biopsy for various reasons.

2. Those patients who are already diagnosed to have cirrhosis or any other liver pathology

3. Patients who are unable to perform light apnea / cardiac patients and patients with respiratory insufficiency.

4. Children less than 2 years.

4. SAMPLING AND CONSENT:

All Thalassemic children from the Department of Haematology; who fulfilled the inclusion criteria were included in the study.

An informed consent was taken from the patient prior to the ARFI scan as per the Institutional Review Board guidelines. The ARFI scans were performed using the Seimens Accuson 2000 USG machine and the mean shear velocity measurements were recorded and entered into the Epidata spreadsheet. The liver biopsy grades and Serum Ferritin values were taken from the clinical workstation.

The consent form and the patient information sheets are attached (APPENDIX 1)

5. TIMING:

The study period was from January 2014 - August 2015.The time period between ARFI liver and liver biopsy was a maximum of 1 month. The ARFI scan was performed prior to the liver biopsy. The primary investigator was blinded from the results of the liver biopsy till the end of the study.

The liver biopsy was performed by interventional radiologists along with Hickman's catheter insertion for the > 15 years age group and for the < 15 years age group it was done by paediatric surgeons after the ARFI scan.

6. VARIABLES:

The various variables studied were patient's age, gender, serum Ferritin, ARFI values and ARFI grades of fibrosis. The ARFI values measured were in metres /sec (m/sec). The patients then underwent liver biopsy after which the grade of liver fibrosis were scored by the ISHAK scoring system. The ARFI grades ascertained were ranging from F1-F4 levels and the liver biopsy results as per the ISHAK grading system were from F0-F6.

The reference values for ARFI elastography were taken from a prior study as 1.18 m/sec, 1.21 m/sec, 1.54 m/sec and 1.94 m/sec for F1, F2, F3 and F4 grades of fibrosis respectively. The sensitivity of these values were found to be 89%, 100%, 97% and 100% and the specificities were found to be 87%,71%,100% and 98% respectively for F1,F2, F3 and F4 grades of fibrosis.(52)

7. STANDARD OPERATING PROCEDURE:

PERFORMING THE ULTRASOUND ARFI SCAN OF LIVER:

Acoustic radiation force Impulse (ARFI) elastography is a shear wave elastography technique. It was performed on the SEIMENS Acuson S 2000 Medical Solutions which combines both conventional B-mode Ultrasound and the "Virtual Touch Tissue Quantification" (VTQ). The VTQ provided is incorporated in the SEIMENS machine which measures the tissue stiffness and generates the shear velocity values.

The transducer generates a pressure wave automatically which propagates in the liver. The sonologist / operator selects the depth at which the liver elasticity is to be evaluated, by placing a region of interest / measuring box (10 mm long x 5 mm wide) in the segment of the liver from which it has to be assessed.

The study is performed with maximum right arm abduction in the supine position usually in the right lobe of liver through the window provided by the intercostal spaces. The segment 5 and 8 of the liver are used commonly for the ARFI elastography avoiding the cardiac motion as far as possible. Patient is advised to hold the breath for a short while following which the shear velocities are obtained from a depth of atleast > 2 cm from the liver surface and not exceeding 5 cm. The values are obtained in m/sec and are displayed on the screen. About 5-10 valid measurements are taken from each segment and the mean and median value is calculated (automatically by the machine), the result being measured in metre/sec (m/s).

The shear wave velocity increases with increasing levels of fibrosis and cirrhosis. The advantage is that both the gray scale image of the liver as well as shear velocity

measurements are obtained which gives an overall idea about the status of liver fibrosis.

8. PERSONNEL:

a. Person performing ARFI and its interpretation:

i. PG resident doing the thesis

b. Person reporting liver biopsy:

i. 2 experienced hepatopathologist with more than 5 years and 20 years experience

9. EQUIPMENT:

1. USG Machine:

USG Machine SEIMENS ACCUSON S 2000 on which the ARFI scans were performed

2. Ultrasound transducer used for the study:

4MHz ultrasound transducer used to perform the ARFI elastography.

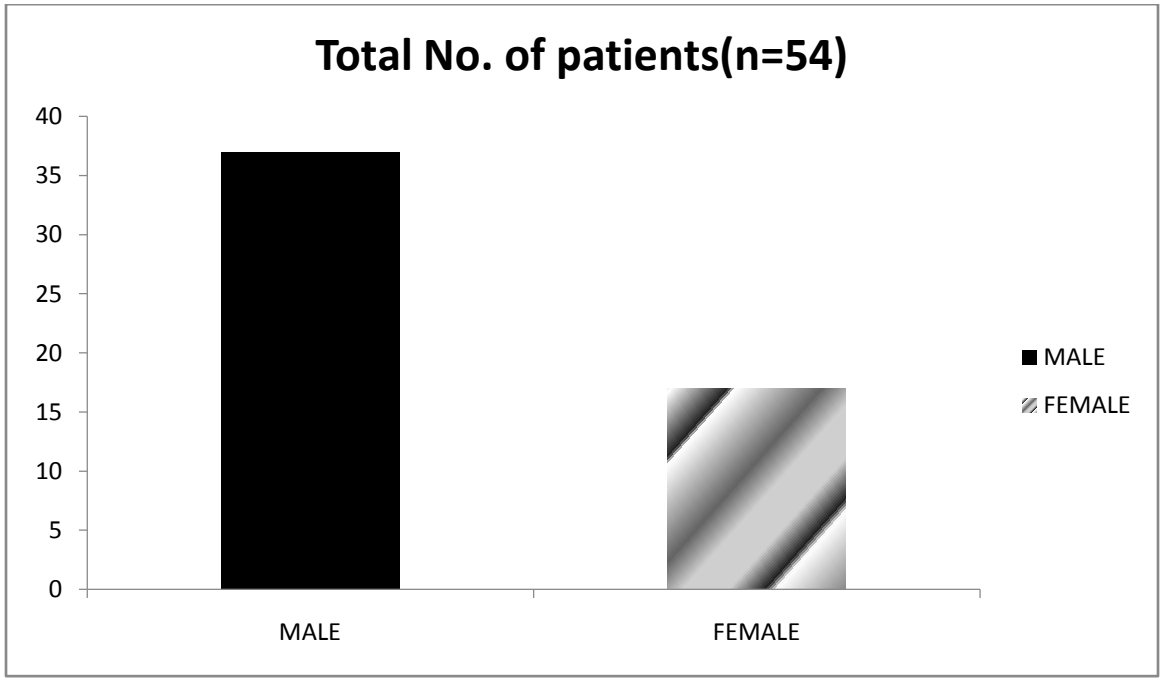
RESULTS:

Total Number of Thalassemic patients: 54

A) Gender:

- Male: 37

- Women: 17



B) Age:

- Average age of the subjects was 8.1 years (minimum 2 years - maximum 20 years)

C) Distribution of number of cases among the various ARFI grades:

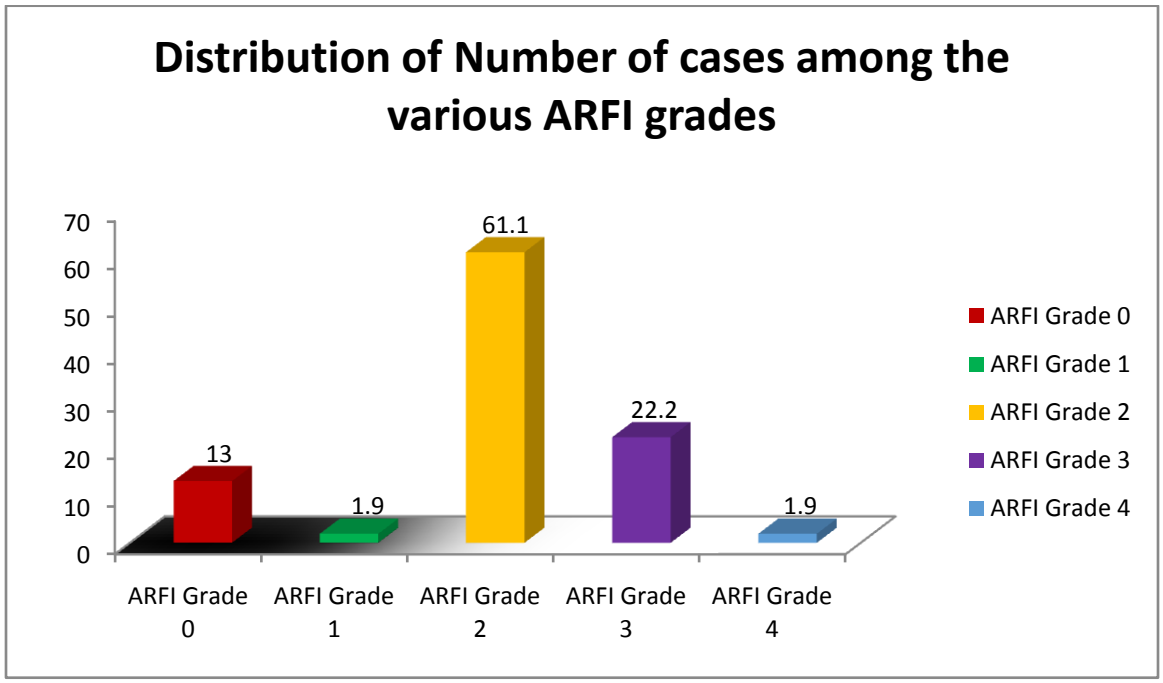
ARFI grade 0(F0): 7 (13%)

ARFI grade 1(F1): 1 (1.9%)

ARFI grade 2(F2): 33 (61.1%)

ARFI grade 3(F3): 12 (22.2%)

ARFI grade 4(F4): 1 (1.9%)



D) Distribution of number of cases among the various liver biopsy grades (ISHAK):

ISHAK grade 0: 7 (13 %)

ISHAK grade 1: 9 (16.7 %)

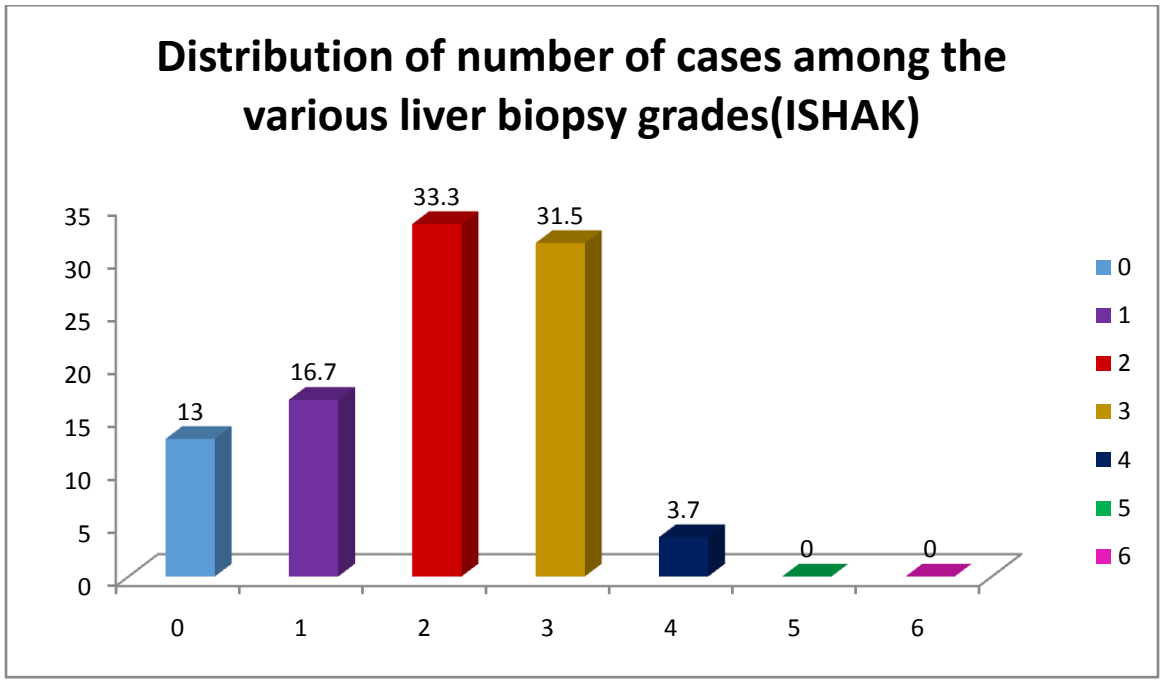
ISHAK grade 2: 18 (33.3 %)

ISHAK grade 3: 17 (31.5 %)

ISHAK grade 4: 2 (3.7 %)

ISHAK grade 5: 0 (0%)

ISHAK grade 6: 0 (0%)



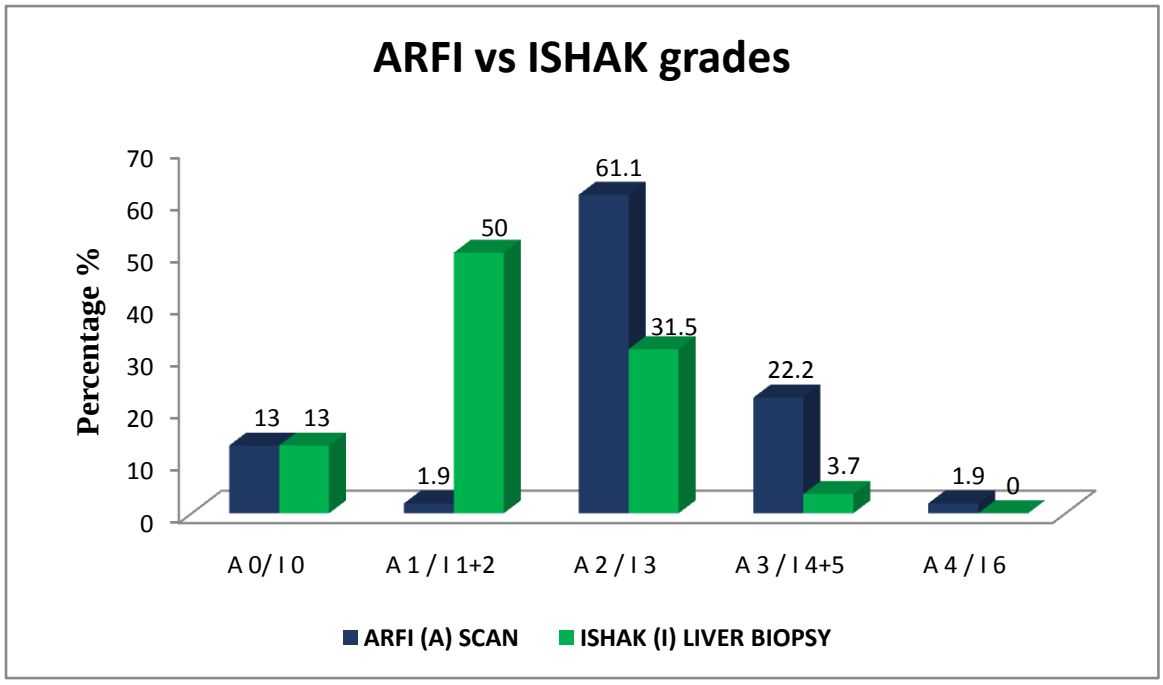
This table below groups the F1 and F2 of ISHAK liver biopsy grades as F1 and F4 and F5 on ISHAK as F3.

Ishak Score; Fibrosis 0-6	METAVIR Score; Fibrosis 0-4
0	0
1-2 (mild)	1
3 (moderate)	2
4-5(severe)	3
6	4

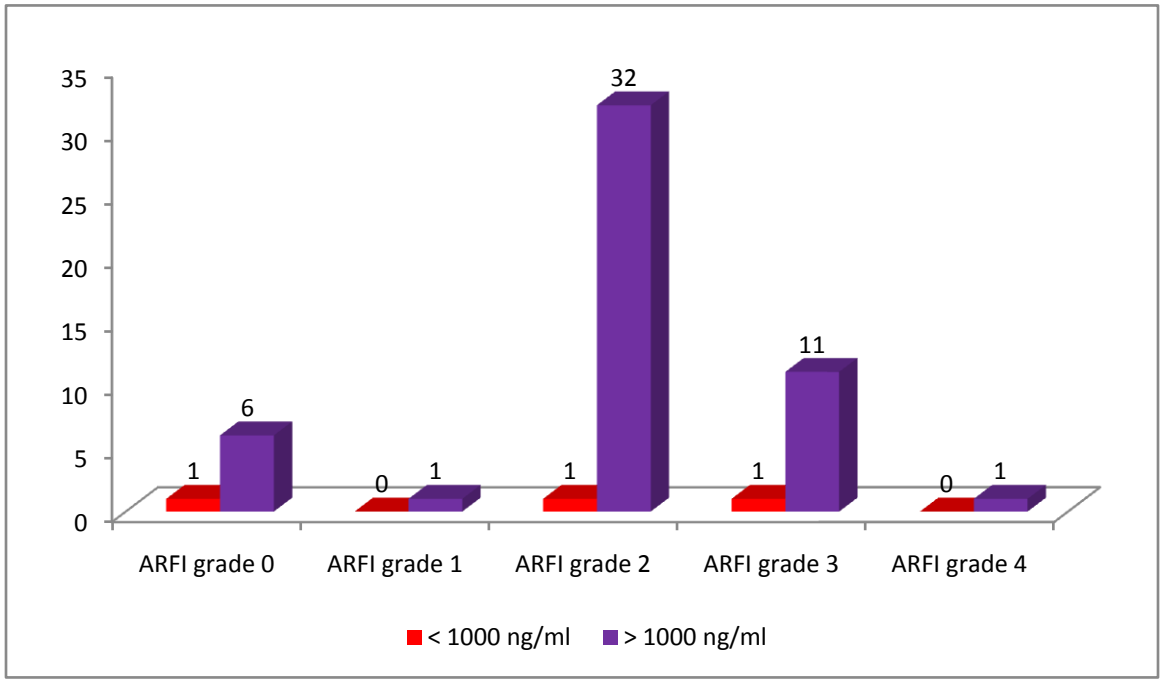
The ARFI grades were compared to ISHAK grades in the following combination according to the relevance of each ARFI grades to that of liver fibrosis on the ISHAK scoring system.

ARFI Grades	ISHAK Grades
F0 (13%)	F0 (13%)
F1 (1.9%)	F1 + F2 (50%)
F2 (61.1%)	F3 (31.5%)
F3 (22.2%)	F4+F5 (3.7%)
F4 (1.9%)	F6 (0%)

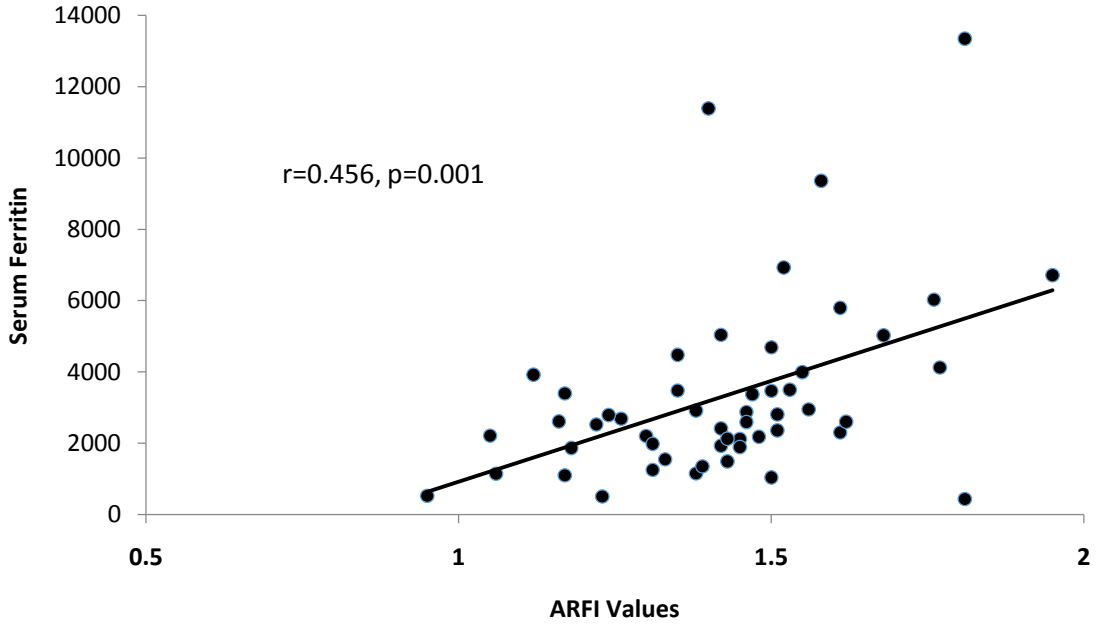
E) ARFI Grades Vs Liver biopsy Grades (ISHAK) comparison:



F) Distribution of number of cases in various ARFI grades in comparison to the serum Ferritin values:



G) Distribution of cases among the various Ferritin values:



This table shows the age, ARFI values and serum Ferritin levels (minimum, maximum and mean values)

	Age of patient	ARFI values(m/sec)	Serum Ferritin (ng/ml)
Mean	8.11	1.4200	3267.53
Median	7.50	1.4250	2600.00
Std. Deviation	4.717	.20381	2509.816
Minimum	2	.95	445
Maximum	20	1.95	1334

H) The diagnostic accuracy of ARFI to pick up fibrosis among the total no of patients was as follows:

	Liver biopsy 123456	**LIVER biopsy 0**	*TOTAL*
ARFI 1234	**41**	**5**	*47*
ARFI 0	**5**	**2**	*7*
TOTAL	*47*	*7*	*53*

Sensitivity - 89%

Specificity – 29%

Positive predictive value (PPV) – 89%

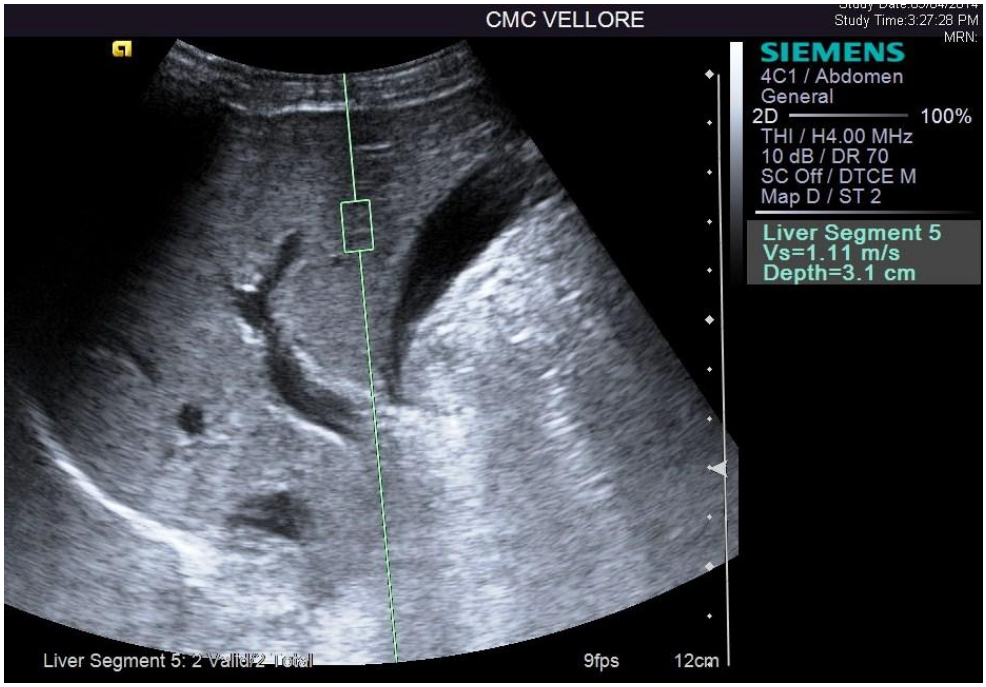
Negative predictive value (NPV) – 29%

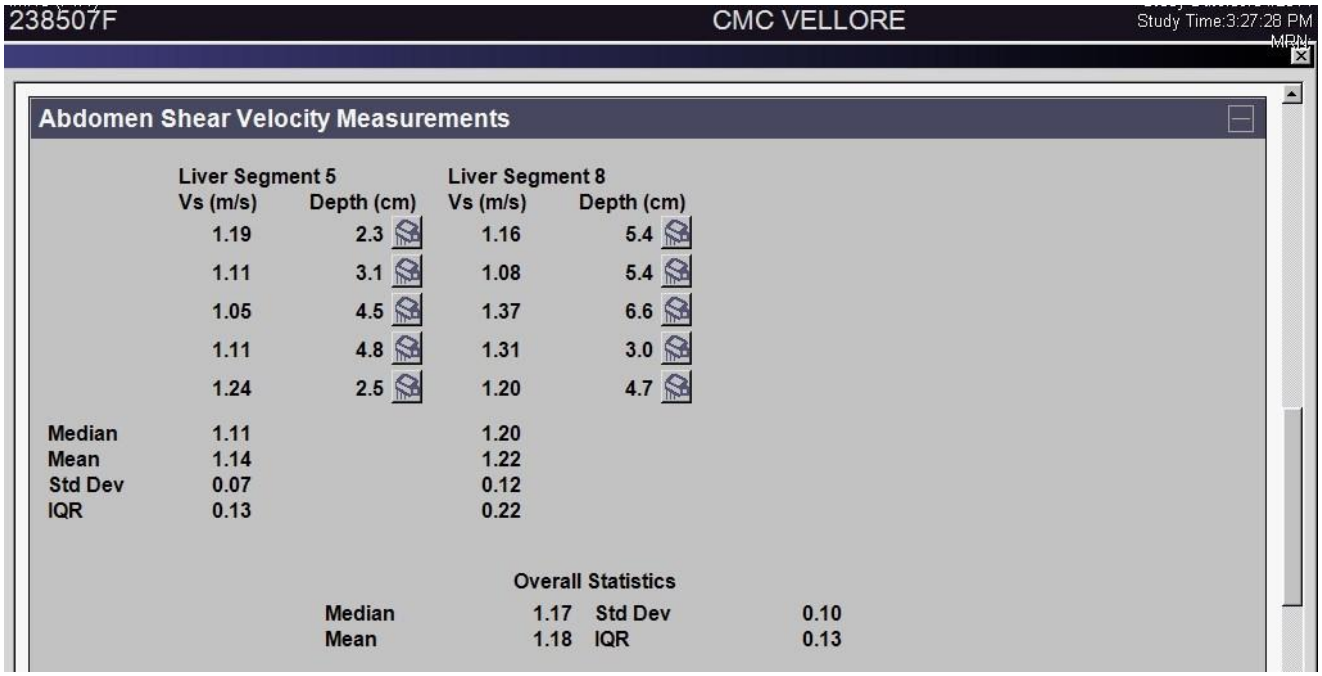
Diagnostic accuracy: 41+2 / 53(total cases) x 100 = 81 % (one sample had only one complete portal tract and so was excluded from the analysis)

Kappa – 18% (agreement)

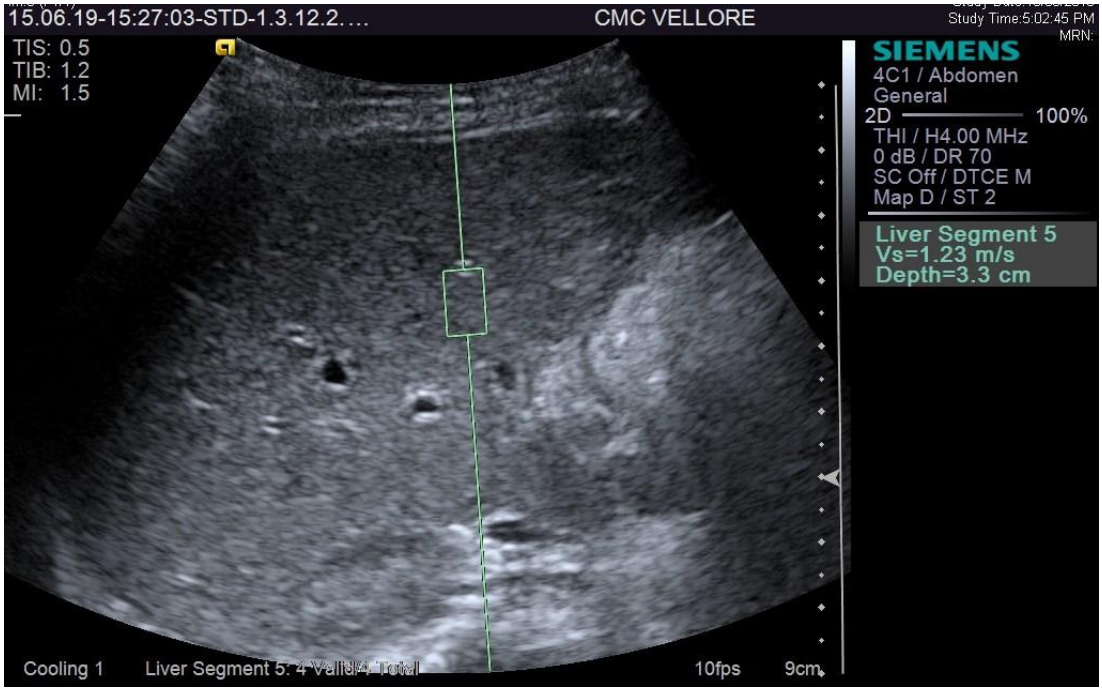
ARFI ELASTOGRAPHY AND VARIOUS GRADES OF FIBROSIS:

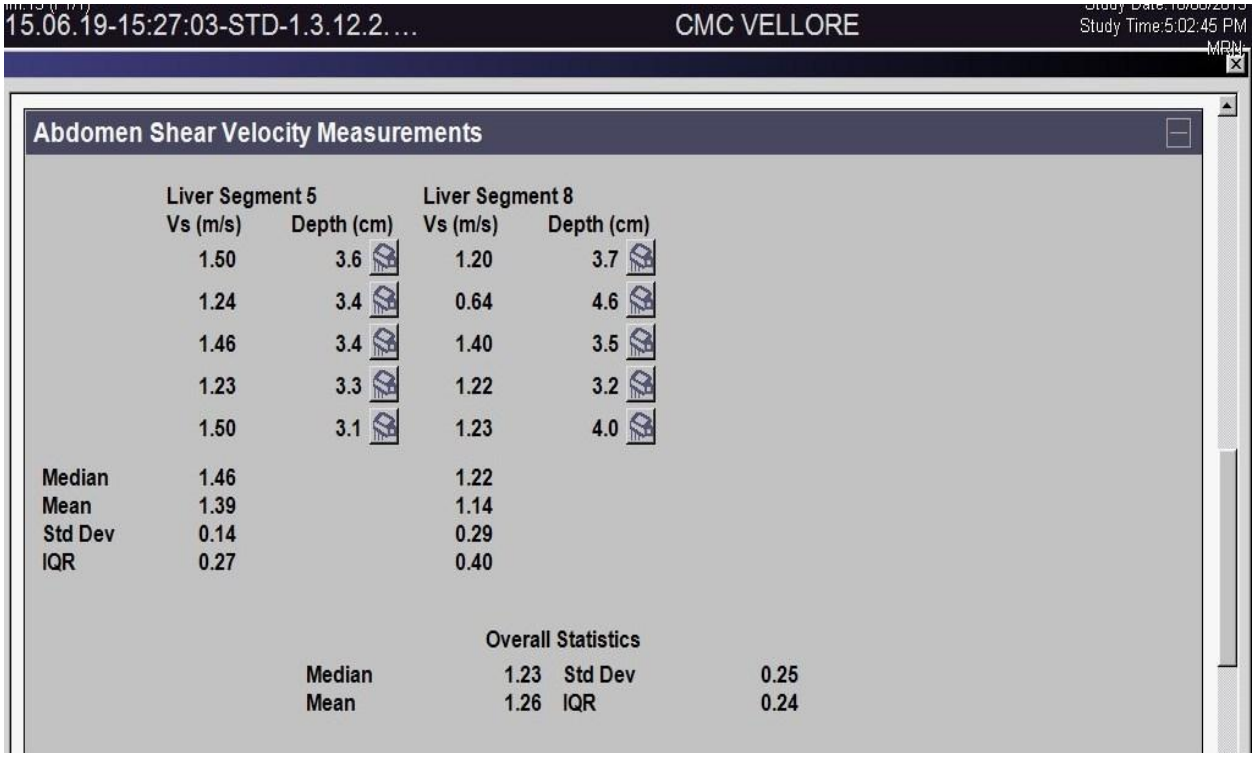
1) ARFI grade 1:



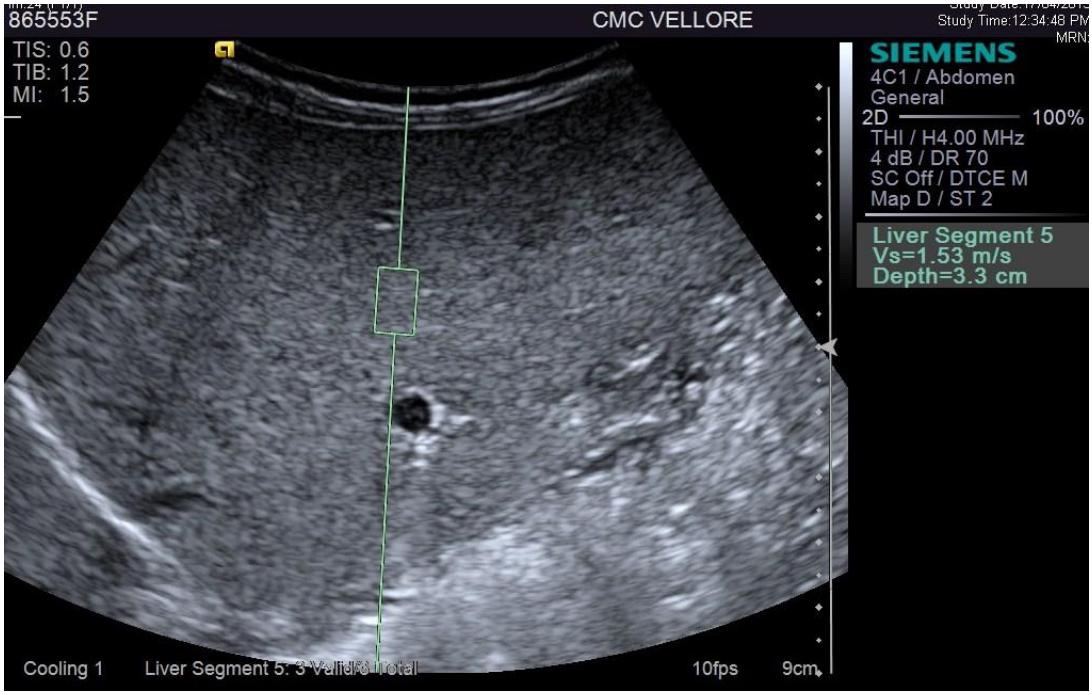


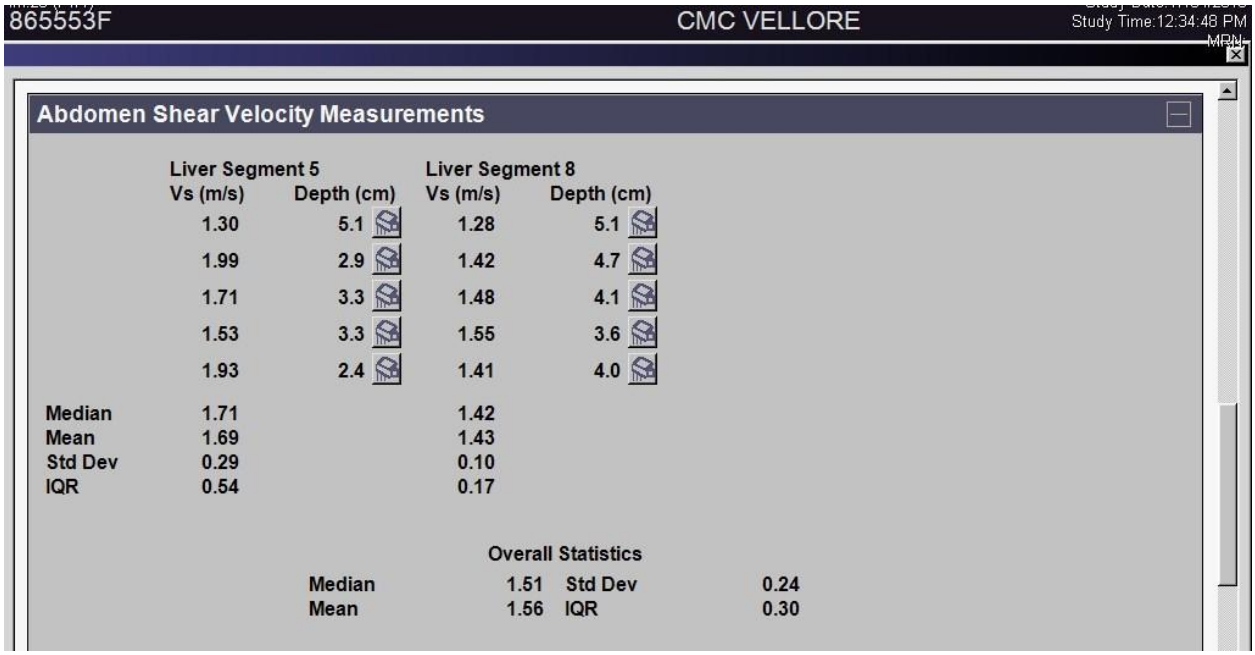
2) ARFI grade 2:



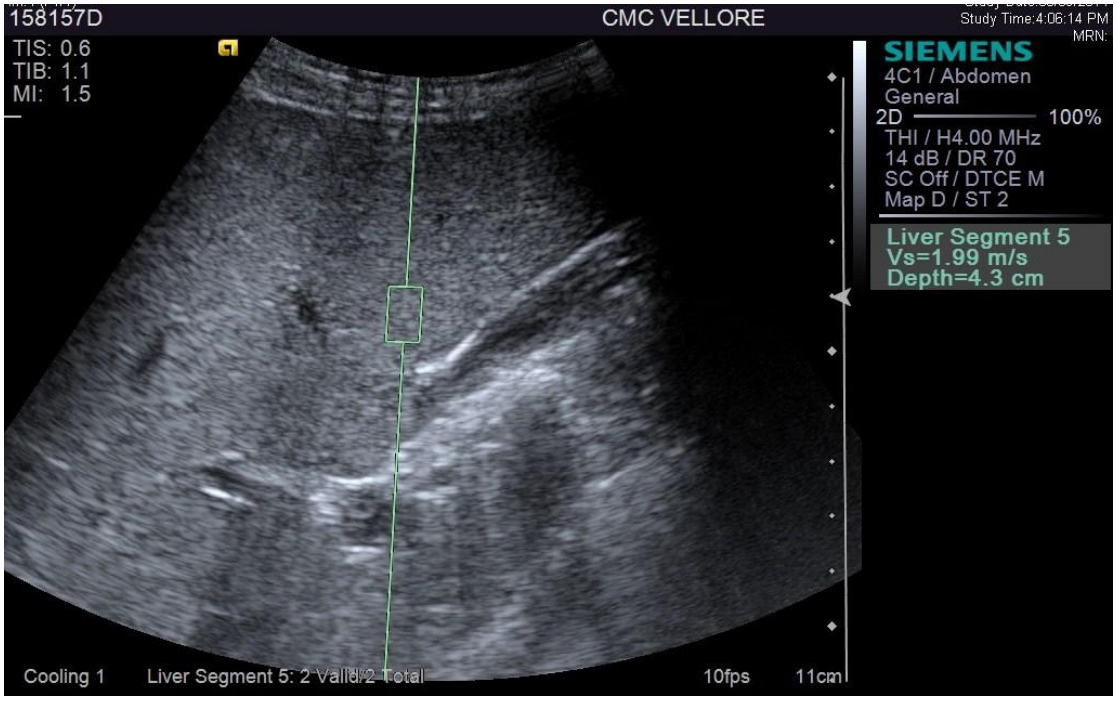


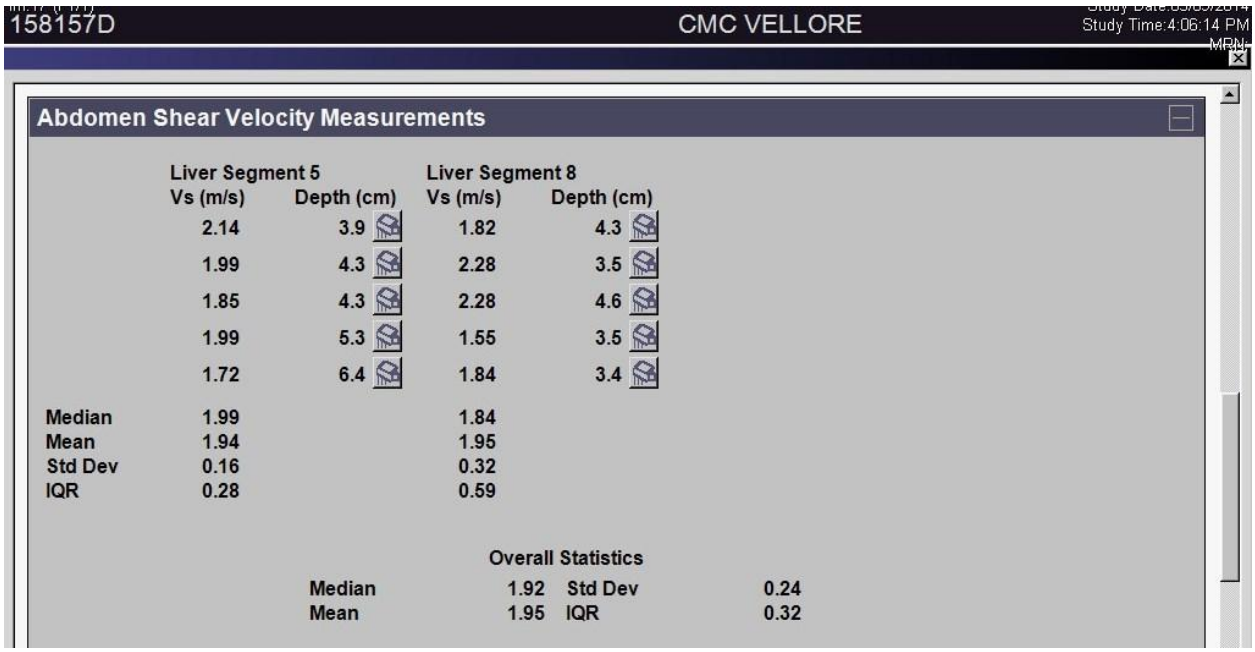
3) ARFI grade 3:





4) ARFI grade 4:



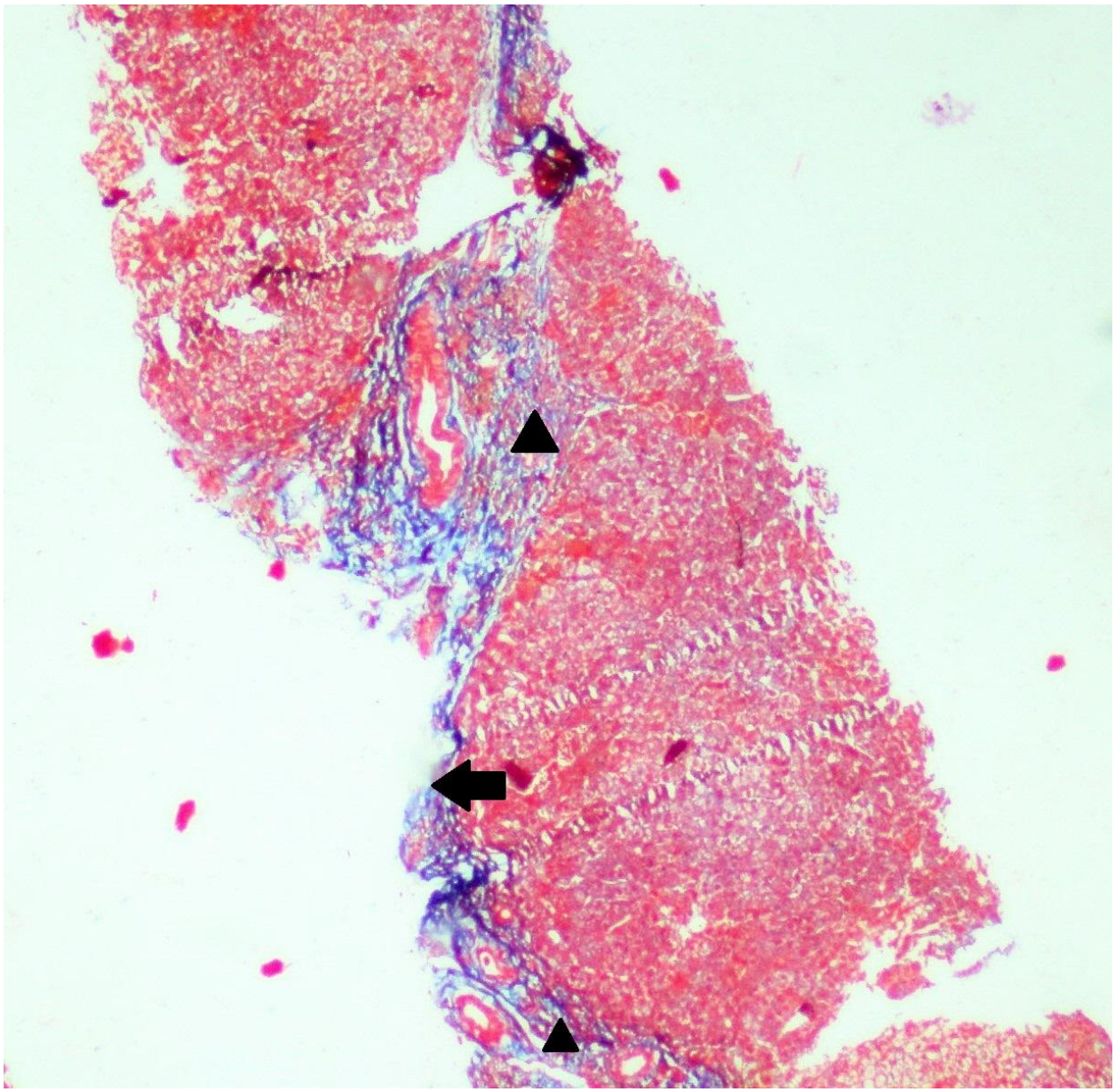


PHOTOMICROGRAPHS OF ISHAK GRADES OF FIBROSIS:

1) F1 / F2 ISHAK:

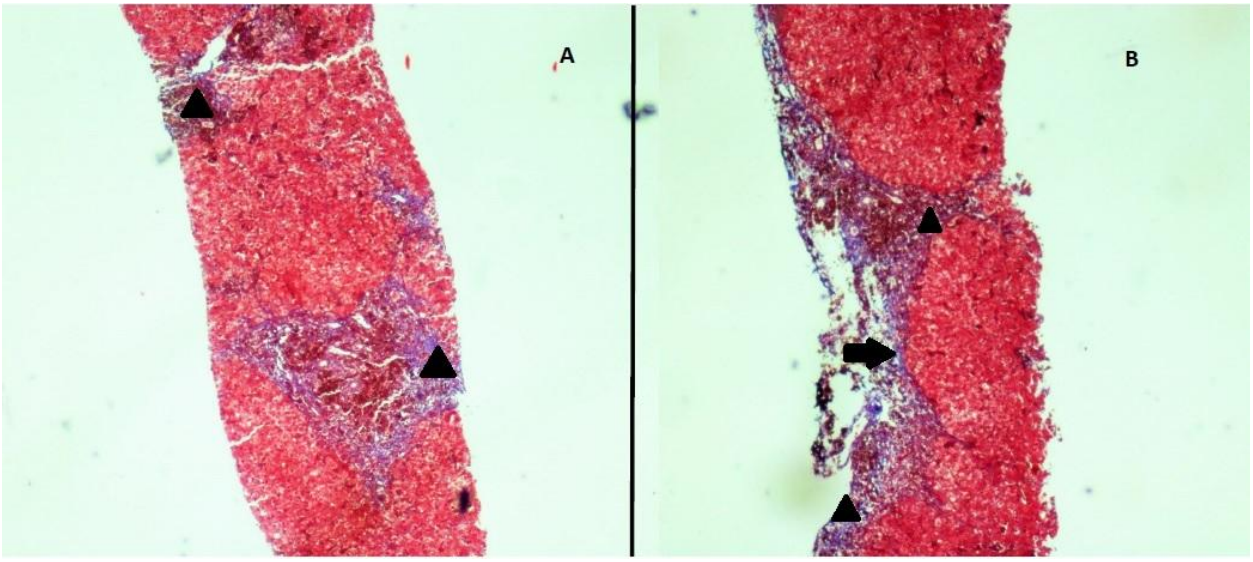
F1 / F2 ISHAK: Micro-photograph (Masson Trichrome stain; 100X) displaying mild portal expansion with slender extensions (arrow) into the adjacent parenchyma

2) F3 ISHAK:



Micro-photograph (Masson trichrome stain; 100X) displaying portal fibrosis (arrow heads) with an occasional portal to portal fibrous linkage (arrow)

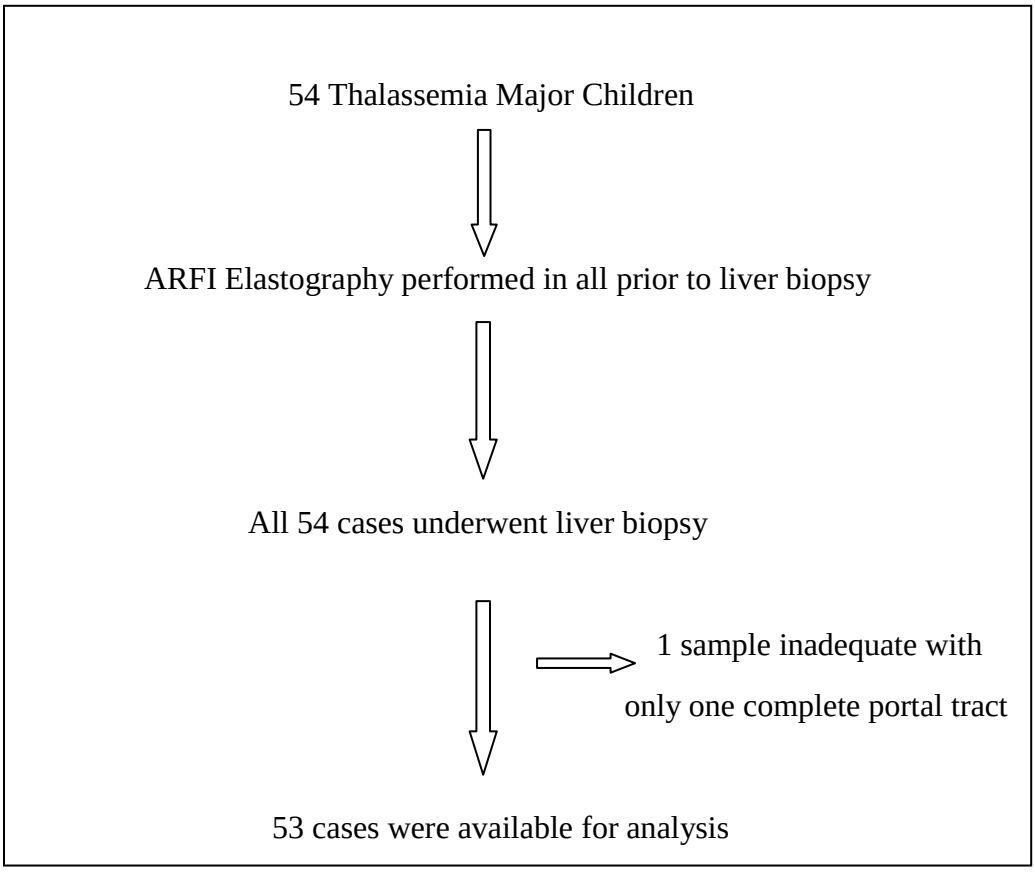
3) F4 ISHAK:



Micro-photographs (Masson trichrome stain; 100X) displaying moderate portal fibrosis (arrowheads in A & B) with portal to portal fibrous linkage (arrow in B)

DISCUSSION:

A total of 54 out of a total of 60 cases were included in this study. Study was performed on a pediatric population within the age group of 2-20 years (mean age 8.1 years). ARFI liver was done prior to the liver biopsy in all these patients.



54 cases underwent ARFI scan and all 54 cases underwent liver biopsy; however there was one insufficient sample with only one complete portal tract which was not included in the statistical analysis. All the patients had a serum Ferritin value test prior to or within few days of the ARFI liver scan.

In this study it was found that ARFI elastography of liver was 89% sensitive with a positive predictive value of 89% in diagnosing liver fibrosis in comparison to the histopathological liver biopsy. It also showed a diagnostic accuracy of 81% for the assessment of liver fibrosis. However it was only 29 % specific in the assessment of fibrosis in this group of Thalassemic patients. There was only 18% agreement (kappa) between the two tests and it did not show any statistically significant correlation between the ARFI scan versus the histopathological grades on liver biopsy (ISHAK); p value being 0.942.The agreement between the two tests in the form of kappa was also 18% which also shows only minor agreement.

ARFI scan had moderate ability in diagnosing > F2 and F3 grades of fibrosis to the corresponding ISHAK histopathological grades rather than the lower grades of fibrosis.

There were a total of 33 patients (61.1%) in F2 grades and about 12(22.2%) patients with F3 grades on ARFI; however the corresponding liver biopsy grades on ISHAK i.e. F3 had 17 (31.5 %) patients and F4 + F5 had 2 (3.7 %) patients respectively.

As there was already available cut off based on which the ARFI study was performed and lack of any normal study population cut-offs for liver fibrosis cannot be assessed in the present study.

About 84% of patients with a serum Ferritin value of > 1000 ng/ml were distributed in the F2 and F3 fibrosis group and there was a moderate correlation between the levels of Serum Ferritin with that of the ARFI grades(r=0.456, p=0.001). This was statistically significant with p value < 0.05.

CONCLUSION:

1) ARFI elastography has a good ability in assessing liver fibrosis with a diagnostic accuracy of 81%, sensitivity and positive predictive value of 89% as compared to histopathological liver biopsy grading in children with Thalassemia; however its sensitivity and specificity is only about 29%.

2) ARFI elastography can be used as good non-invasive test for assessing patients with liver fibrosis; however further studies need to be done to standardize the same.

3) Serum Ferritin levels show moderate correlation with ARFI grades and higher values of serum Ferritin are distributed among the higher ARFI grades of fibrosis.

LIMITATIONS:

1. Sample size**:** The calculated sample size was 60; however due to the rate of flow of patients (about 2-3 cases of Thalassemia patients are admitted per month for bone marrow transplantation); 54 cases were done. Further recruitment of cases is still ongoing.

2. There is no available literature from India or elsewhere on the usefulness of ARFI in Thalassemia patients as compared to liver biopsy hence there was no study available for comparison.

3. Cut-off values for ARFI were taken from adult population values in already published journals; there is no study available for reference in the pediatric age group.

TECHNICAL FACTORS:

1. As the study was conducted on a pediatric age group short breath holding was a problem. Hence, some of the values could have been erroneously taken.

2. There is no study available which quotes the use a smaller transducer for performing ARFI in children; hence narrow intercostal space with a large probe could have also lead to erroneous shear velocity measurements in this group of patients.

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ANNEXURES:

Consent forms

Department of Radiodiagnosis, Christian Medical College, Vellore

Study Title: ***Assessment of role of ARFI Scan in assessment of liver fibrosis in patients planned for Bone Marrow transplantation***

PATIENT INFORMATION SHEET

You are being requested to participate in a study to see if ARFI scan of the liver will be able to assess fibrosis of the liver. At present the knowledge of ARFI scan to pick up fibrosis is limited to cases of Non alcoholic fatty liver disease, Chronic liver disease etc. The knowledge of this test for the assessment of fibrosis in Thalassemic patients is not available. Hence we hope to find out whether this ARFI scan will assess fibrosis adequately in patients planned for liver biopsy prior to their Bone Marrow Transplantation

What additional tests do I have to go through if I take part in this study?

If you take part in this study, your ARFI scan will be done as a routine test by the primary investigator as prescribed by your clinician. You will not have to pay any additional amount.

In addition, liver biopsy will be done for all the Thalassemic patients going for bone marrow transplantation and the tissue will be sent to Pathology department to be looked under microscope. This biopsy will not be done by the primary investigator.

Does ARFI scan have any side effects?

ARFI scan does not have any harmful radiation. It is a painless non- invasive study. We will be doing it the same way as you would have it if you were not included in this study.

If I take part in this study, what will I have to do?

If you agree to participate in this study, there will be no change in the other investigations and treatment that you will be receiving. You will be expected to come for the ARFI as advised by your doctor. No additional blood tests will be done as a part of this study.

Can I withdraw from this study after it starts?

Your participation in this study is entirely voluntary and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your usual treatment at this hospital in any way.

What will happen if I develop any study related injury?

This scan does not involve harmful radiation and it is completely non invasive. So, we do not expect any procedure related injury. However you can immediately report to us.

Will I have to pay for the additional tests?

You will **not** be charged additional amount for this scan. All other investigations, as requested by your doctor will continue in the usual manner. How much you pay for these investigations will not change and this has nothing to do with your participation in this study.

What happens after the study is over?

You may or may not benefit from this study. Once the study is over, we will analyze the results and come to a conclusion and we will be able to use these results in helping other patients with Thalassemia with suspected liver fibrosis to undergo this non invasive test rather than liver biopsy for the assessment of fibrosis prior to Bone marrow transplantation

Will my personal details be kept confidential?

The results of this study will/may be published in a medical journal but you will not be identified by name in any publication or presentation of results. However, your medical record **may** be reviewed by doctors associated with the study, without your additional permission.

If you have any further questions, please contact Dr. Priyanka Mohapatra (Tel: 0416 228- 3012/2027/3609) between 8am & 4:30pm from Monday to Friday and from 8am to 12:30pm on Saturday or you can email your queries to priyanka.rini@gmail.com / mohapatrapriyanka2005@gmail.com

Department of Radiodiagnosis, Christian Medical College, Vellore

CONSENT TO TAKE PART IN ARFI LIVER FIBROSIS IN THALASSEMIA STUDY

Study Title: ***Assessment of role of ARFI Scan in assessment of liver fibrosis in patients who are planned for Bone Marrow transplantation***

Study Number:
Patient's name:
Hospital No:
Date of Birth / Age (in years):

I _____, patient, father / mother of the patient named _____ declare that I have read / been read to the information sheet provided to me regarding this study and have clarified any doubts that I had. [ ] (Please tick boxes)

I also understand that my participation/ my child's participation in this study is entirely voluntary and that I am free to withdraw permission to continue to participate at any time without affecting patient's usual treatment or legal rights [ ]

I understand that study staff and institutional ethics committee will not need my permission to look at patient's health records if I/ my child withdraw/ withdraws from the trial. I agree to this access [ ]

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s) [ ]

I understand that patient's identity will not be revealed in any information released to third parties or published [ ]

I voluntarily agree to take part in this study [ ]

Name:
Patient/Relation to the patient:
Signature/thumb impression:
Date:

Name of witness:
Relation to participant:

Date:

Data sheet

id	name	hospno	age	sex	datearf	arfvalue	arfgrades	datebio	biograde	ferritin
1	AANVI SETHIA	480801F	8	2	14/05/2014	1.61	3	02/07/2014	3	2312
2	ABHRAJIT GHOSHAL	808106F	2	1	01/06/2015	1.17	0	08/06/2015	0	1110
3	ADEEBA FATIMA	031823G	2	2	03/07/2015	1.3	2	13/07/2015	2	2212
4	ALFISHA	781873F	8	2	07/08/2014	1.42	2	01/09/2014	0	5048
5	AMOGH SHARMA	723499F	5	1	03/01/2015	1.31	2	14/01/2015	missing	1985
6	ANKIT KUMAR	249740F	12	1	30/06/2014	1.4	2	21/07/2014	2	11390
7	ASHA ARVINDBHAI KATARMAL	669747F	20	2	26/03/2014	1.81	3	03/04/2014	3	13345
8	BHUBESH CHANDNANI	874338F	11	1	20/01/2015	1.5	2	02/02/2015	3	4698
9	DEVANSH GABA	793205F	4	1	24/01/2015	1.38	2	09/02/2015	2	1164
10	DHRUV	480954F	7	1	02/01/2015	1.45	2	19/01/2015	1	2120
11	FATHIMATH SHIURA SUJOON	595578D	8	2	18/06/2014	1.45	2	30/06/2014	3	1892
12	GAURAV MOTIANI	853174F	8	1	04/03/2015	1.12	0	30/03/2015	2	3925
13	GIRIDHAR	784151D	5	1	18/06/2015	1.46	2	24/06/2015	3	2887
14	HARIHARANSUDHAN	820841C	14	1	30/09/2014	1.46	2	01/11/2014	2	2596
15	HAWARAA	741558F	19	2	13/02/2014	1.35	2	10/02/2014	2	3488
16	HIMANSHU ASHWIN POPAT	492710F	14	1	02/08/2014	1.51	2	28/08/2014	2	2811
17	HIMANSHU SAHOO	805608F	9	1	24/10/2014	1.5	2	05/11/2014	4	3477
18	ISHANT	238507F	9	1	09/04/2014	1.18	1	28/04/2014	2	1866
19	KEVIN MEHULKUMAR THAKKAR	963879D	5	1	11/10/2014	1.05	0	12/11/2014	2	2221
20	KISHAN	640900F	4	1	29/09/2014	1.42	2	20/10/2014	3	1933
21	MAHIR NIKHIL SHAH	126831G	3	1	18/06/2015	1.26	2	29/06/2015	3	2699
22	MANIKANTA A.S.S.	045355G	10	1	07/07/2015	1.52	2	27/07/2015	3	6929
23	MEET BHAMBANI	890498F	5	1	26/08/2014	1.35	2	17/09/2014	2	4477
24	MEHAK IBRAHIM	314024F	7	1	12/05/2014	1.61	3	26/05/2014	1	5799
25	MITHUN	001349G	3	1	24/04/2015	1.33	2	20/05/2015	1	1558
26	MOHAMMAD AHNAF	421858F	3	1	29/09/2014	1.51	2	08/10/2014	0	2362
27	MOHAMMAD MUSHARAF	785335F	9	1	31/03/2014	1.81	3	14/04/2014	3	445
28	MONIK VIJAYKUMAR ZADE	245134F	7	1	02/09/2014	1.31	2	29/09/2014	3	1262
29	NAVADEEP	052042F	5	1	22/02/2014	1.5	2	17/03/2014	1	1045
30	NIHARIKA NAGDEV	662479F	6	2	28/11/2014	1.24	2	15/12/2014	1	2791
31	NIKUNJ GOPAL BHANUSALI	346183F	8	1	29/01/2015	1.55	3	09/02/2015	0	3996
32	NISHTHA P MULCHANDANI	268774F	5	2	07/08/2014	1.17	0	08/09/2014	2	3407
33	PARTH ABROL	849302F	8	1	27/06/2014	1.76	3	07/07/2014	3	6037
34	PRIYANSHU CHETAN	047314G	12	1	08/03/2015	1.39	2	09/03/2015	3	1362

	VYAS									
35	SAEED NISAR	155590G	4	1	20/02/2015	1.43	2	04/03/2015	0	1491
36	SANIL KUMAR JAIN	158157D	17	1	03/09/2014	1.95	4	03/10/2014	4	6713
37	SHIVAM BHANOT	419001F	5	1	03/05/2014	1.47	2	21/05/2014	1	3373
38	SIMRAN OTWANI	310897F	11	1	28/03/2014	0.95	0	07/04/2015	2	536.4
39	SRIRAM	627005B	18	1	22/02/2014	1.42	2	06/03/2014	2	2415
40	TAHURA SALEHA	326138F	13	2	03/04/2014	1.43	2	05/05/2014	3	2125
41	THOLU PAVAN	751315F	7	1	30/09/2014	1.06	0	30/10/2014	1	1155
42	TRISHA SARDAR	007769G	8	2	09/04/2015	1.53	3	20/04/2015	3	3500
43	TUSHAR DHARMALE	144108G	10	1	02/06/2015	1.77	3	24/06/2015	2	4133
44	VAISHNAVI MEGHANA R	476246F	2	2	05/06/2015	1.23	2	17/06/2015	0	518
45	VAMSITHARAN	091466C	14	1	21/03/2015	1.62	3	08/04/2015	2	2604
46	VANDIT WADHWANJ	936054B	16	1	17/11/2014	1.58	3	19/11/2014	3	9363
47	VANSHIKA BHANUSHALI	002916G	4	2	29/01/2015	1.38	2	16/02/2015	1	2917
48	VIDHI SAHU	865553F	4	2	17/04/2015	1.56	3	29/04/2015	3	2957
49	VIDIT GUPTA	612021F	8	2	04/12/2014	1.48	2	05/01/2015	2	2181
50	VAISHNAVI N	825004F	2	2	23/07/2015	1.16	0	03/08/2015	0	2613
51	PURVESH	010770G	3	1	29/07/2015	1.22	2	17/08/2015	2	2536
52	SAHIL MANOJ NAGRANI	818470F	16	1	05/08/2015	1.68	3	14/08/2015	3	5026
53	ANUSHKA SAMUI	456300D	7	2	08/08/2015	1.39	2	17/08/2015	2	1955
54	NENAVATH VED AASHISH BHANU	122036F	4	2	24/08/2015	1.21	2	07/09/2015	1	1686